ANNEX B

Original version January 1997, revised in March 2010

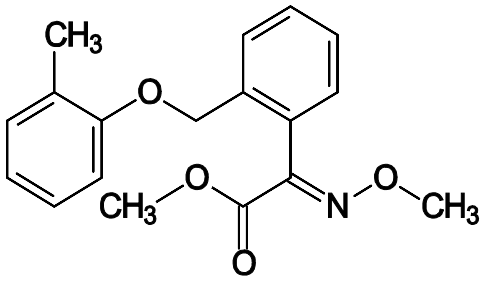
Kresoxim-methyl

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Introductory remarks: Several batches have been used in the studies discussed below. There seems no reason to believe that these are sufficiently different to have an impact on the results. The substance also seems sufficiently stable.

ring A ring B



position of ^{13}C label : CH_3-O-^{13}CO-^{13}C=N-O-CH_3

Kresoxim-methyl = Strobilurin analog = BAS-490F = Reg.n°.242009

Studies in rat:
- Study of the biokinetics of [^{14}C]-Reg.n°-242009 ([^{14}C]-BAS 490 F) in rats (Gans et al. 1994)
- Amendment on the study of the biokinetics of [^{14}C]-Reg.n°-242009 ([^{14}C]-BAS 490 F) in rats (Gans et al. 1995a)
- Study of the biokinetics of [^{14}C]-Reg.n°-242009 ([^{14}C]-BAS 490 F) in rats (Gans et al. 1995b)
- [^{14}C] - Reg.n°-242009 : quantitative whole-body autoradiography following oral administration to the rat (Whitby and Hopkins, 1993)
- The metabolism of [^{14}C] -Reg.n°-242009([^{14}C]- BAS 490 F) in rats (Kohl,1994), addendum 1 (Kohl,1995) and addendum 2 (Kohl,1998).

Guidelines :
Experimental protocol of the 3 studies in rats complies with test method B Directive 87/302/EEC, despite the lack of toxicological data.
The studies are GLP.
Material and methods :
Different formulations of kresoxim-methyl, were prepared as described in table 5.1.1. Male and female Wistar rats (Chbb-THOM-(SPF)) were dosed orally with 50 or 500 mg/kg bw or i.v. treated with 5mg/kg bw. The study designs are described in table B.6.1.2

Table B.6.1-1: Characteristics of the different formulations used.

substance	position of label	vehicle	specific activity	chemical purity (%)	radiochemical purity (%)
^{14}C-kresoxim-methyl	ring A	oral suspension in tylose 0.5% and Pluriol, Miglyol or Cremophor	4.41 MBq/mg	> 98	>98
^{14}C-kresoxim-methyl	ring B	-i.v.solution: NaCl:0.9% -oral suspension in tylose 0.5% and Pluriol, Miglyol or Cremophor	3566 MBq/g	> 97	>98
^{13}C-Reg.n°-273873	(methoxy imido)acetic acid methylester	oral suspension in tylose 0.5% and Pluriol, Miglyol or Cremophor.		>99	-
kresoxim-methyl	unlabelled	oral suspension in tylose 0.5% and Pluriol, Miglyol or Cremophor.		> 94	-

Table B. 6.1-2 : Study designs

experimental series as identified in the reports	number animals used ♂	♀	route	dose (mg/kg bw)	dosing frequency	position of label
blood/plasma	Study of the biokinetics of [^{14}C]-Reg.n°-242009([^{14}C]-BAS 490 F) in rats (Gans et al. 1994)					
6	5	5	oral	500	1	ring B
7	5	5	oral	50	1	ring B
8	5	5	oral	500	1	ring B
tissue distribution	Study of the biokinetics of [^{14}C]-Reg.n°-242009([^{14}C]-BAS 490 F) in rats (Gans et al. 1994)					
9	12	12	oral	50	1	ring B
	Study of the biokinetics of [^{14}C]-Reg.n°-242009([^{14}C]-BAS 490 F) in rats (Gans et al. 1995b)					
10	12	12	oral	500	1	ring B
11	0	12	oral	50	1	ring B
whole body auto-radiography	[^{14}C]-Reg.n°-242009: quantitative whole-body autoradiography following oral administration to the rat (Whitby and Hopkins, 1993)					
	5	5	oral	50	1	ring B
metabolism	The metabolism of [^{14}C] -Reg.n°-242009([^{14}C]- BAS 490 F) in rats (Kohl,1994)					
A	5	5	iv	5	1	ring B
B	5	5	oral	50	1	ring B
C	5	5	oral	50	14+1	unlabelled+1x ring B
D	5	5	oral	50	1	ring B
DX	10	10	oral	500	1	ring B
DY	5	5	oral	500	1	ring A
R	4	4	oral	50	1	ring B
S	4	4	oral	500	1	ring B
V	3	3	oral	50	1	ring B
W	3	3	oral	500	1	ring B
balance/excretion	Study of the biokinetics of [^{14}C]-Reg.n°-242009([^{14}C]-BAS 490 F) in rats (Gans et al. 1994)					
1 A	5	5	oral	500	1	ring A
1 B	5	5	oral	500	1	ring B
2	5	5	oral	50	1	ring B
3	5	5	i.v.	5	1	ring B
4	5	5	oral	50 50	14 + 1	unlabelled ring B
5 (metabolism study)	10	10	oral	500	1	ring B + ^{13}C enriched mat.
excretion via bile	Study of the biokinetics of [^{14}C]-Reg.n°-242009([^{14}C]--BAS 490 F) in rats (Gans et al. 1994)					
10	4	4	oral	50	1	ring B
11	4	4	oral	50	1	ring B

Findings :

- Absorption:
Plasma and blood levels of radioactivity (ring B-^{14}C)were measured after single oral administration.
In rats exposed to a single oral low or high dose, plasma concentration reached respectively a maximum value after 1 h or 8 h post dosing . The concentrations declined within 72 h .

Lower concentrations of radioactivity were found in blood indicating that major parts of the radioactivity are in plasma and not bound to cellular blood constituents. A similar decline of the radioactivity with time is found for blood as for plasma. Blood samples were not analysed for the amount of the parent compound. (table B.6.1-3)

Table B. 6.1-3: Mean plasma and blood levels after a single oral administration

	µg Eq/g blood or plasma							
Sex	**male**				**female**			
Dose	**50 mg/kg**		**500 mg/kg**		**50 mg/kg**		**500 mg/kg**	
time (h)	**plasma**	**blood**	**plasma**	**blood**	**plasma**	**blood**	**plasma**	**blood**
0.5	1.55	0.42	2.38	0.3	2.58	0.39	3.19	0.45
1	1.59	0.30	2.53	0.5	2.28	0.47	2.99	0.86
2	1.30	0.26	2.19	0.55	1.68	0.38	2.78	0.89
4	1.39	0.29	2.66	0.65	1.44	0.54	3.05	0.83
8	1.51	0.26	3.36	0.72	1.54	0.36	3.92	1.33
24	0.40	0.14	0.92	0.28	0.47	0.19	0.75	0.36
48	0.16	0.00	0.33	0.31	0.08	0.21	0.19	0.38
72	0.12	0.02	0.19	0.76	0.03	0.00	0.11	0.09
96	0.03	0.00	0.19	0.95	0.07	0.00	0.18	0.00
120	0.03	0.00	0.23	0.06	0.02	0.00	0.06	0.00

Table B.6.1-4 : Pharmacokinetic parameters of radioactivity (ring B-^{14}C) after single oral administration

dose (mg/kg b.w.)		**C_{max} (µg/g)**	**T_{max} (h)**	**t/2 (h)**	**AUC (µg × h/g)**
50	♂	1.59	**1**	**19.1**	**36.88**
	♀	2.58	**0.5**	**16.9**	**36.22**
500	♂	3.36	**8**	**30.5**	**85.94**
	♀	3.92	**8**	**22.1**	**76.49**

The results presented in table B.6.1.4 show that absorption is rapid as suggested by the plasma C_{max} obtained after 0.5 to 1 h. However, the tissue distribution of radioactivity shows that absorption of kresoxim-methyl is low : most of the administered radioactivity was confined within the gastro-intestinal tract (table B. 6.1.5)

Increasing in both sexes the dose level by a factor of about 10 resulted in an increase of the AUC-values by a factor
of about 2.3 for male and 2.1 for female indicating that the process of absorption from the gastro-intestinal tract was saturated at the high dose level.

- Distribution of radioactivity as a function of time:

Tissue concentrations of radioactivity (ring B-^{14}C) was measured
(i) at 0.5h, 8h or 24h after single oral administration of 50 mg/kg bw, and
(ii) at 8h, 20h, 24h or 96h after single oral administration of 500 mg/kg bw

In blood cells, heart, spleen, muscle, brain and bone, levels are <1 µgEq/g tissue.
The highest tissue concentrations were found in the GI tract content, liver, kidney, plasma and adrenal glands.

Table B.6.1-5a : Tissue distribution of radioactivity (50 mg/kg b.w.) as a function of time

	Tissue concentrations of radioactivity (ring B-^{14}C) mg eq/g tissue					
h after administration	**0.5**		**8**		**24**	
Tissue	♂	♀	♂	♀	♂	♀
plasma	**1.44**	1.44	<1	<1	<1	<1
lung	<1	1.21	1.57	<1	<1	<1
kidney	**6.27**	6.29	3.78	<1	1.02	<1
adrenal gland	<1	2.46	1.40	<1	<1	<1
fat tissue	<1	1.68	1.51	<1	<1	<1
thyroid	<1	1.57	<1	<1	<1	<1
pancreas	<1	4.84	<1	<1	<1	<1
stomach contents	3125.07	2823.8	394.9	25.15	47.33	<1
stomach	237.11	234.21	37.78	4.03	4.6	2.51
gut content	377.52	366.5	513.16	463.57	119.5	16.35
gut	41.08	27.89	57.15	48.8	16.88	5.42
liver	**6.3**	4.88	4.62	1.89	1.36	<1
skin	<1	0.49	<1	1.24	<1	<1
carcass	<1	0.91	5.32	11.56	1.18	<1

At the high dose, apart from the residual radioactivity in the GIT and in the stomach (and its content), high levels at 8h post-dose were measured in the kidney, adrenal gland, ovary/uterus and liver. At 96h, the highest levels were located in the adrenal gland and the ovary / uterus. Expressed as % of administered dose, residues at 96h were all <0.5%, and mostly 0-0.02% of the dose. The highest radioactivity levels were in the skin (0.25-0.43%) and in the carcass (0.31-0.38%) (see Table B.6.1-5b)

Table B.6.1-5b : Tissue distribution of radioactivity (500 mg/kg b.w.) as a function of time

	Tissue concentrations of radioactivity (ring B-^{14}C) µg eq/g tissue							
h after administration	**8**		**20**		**24**		**96**	
Tissue	♂	♀	♂	♀	♂	♀	♂	♀
RBC	3.33	2.26	0.69	1.75	2.33	2.56	0.99	1.24
plasma	22.19	25.68	4.24	3.92	6.77	4.05	0.91	0.93
lung	19.18	18.99	2.86	3.21	7.69	7.93	4.58	4.80
heart	10.66	11.44	1.42	1.46	6.36	6.84	4.79	5.89
spleen	11.92	11.90	9.45	1.71	10.10	8.66	5.43	7.33
kidney	**57.32**	**59.23**	**10.19**	**14.56**	**20.35**	**10.88**	**3.05**	**3.16**
adrenal gland	**53.91**	**56.17**	**4.89**	**4.15**	**36.60**	**36.12**	**36.15**	**33.50**
testes	7.95	-	0.83	-	4.22	-	2.63	-
ovary / uterus	**-**	**56.73**	**-**	**16.07**	**-**	**62.92**	**-**	**37.89**
muscle	14.71	8.53	1.16	1.92	23.45	21.09	30.03	16.17
brain	1.46	2.52	0.18	0.20	0.43	0.32	0.21	0.22
fat tissue	12.78	48.76	19.81	15.97	21.16	17.91	9.61	8.94
bone	6.62	8.82	0.71	0.79	6.57	7.39	7.67	5.21
thyroid	29.72	12.62	1.86	1.79	10.99	11.90	8.92	10.42
pancreas	27.96	213.28	15.52	36.78	43.15	65.18	14.10	11.09
stomach contents	*14090*	*10072*	*99.40*	*718.45*	*545.97*	*203.17*	*6.43*	*17.34*
stomach	*2348*	*1943*	*40.95*	*123.78*	*128.20*	*104.69*	*15.66*	*47.61*
gut content	*5625*	*6632*	*862.65*	*1592*	*590.79*	*460.74*	*17.80*	*16.95*
gut	*726.72*	*681.95*	*203.06*	*349.00*	*381.89*	*375.57*	*24.53*	*21.47*
liver	**58.01**	**63.33**	**13.43**	**13.68**	**17.30**	**13.03**	**3.85**	**4.01**
skin	21.81	22.22	3.20	2.17	14.62	12.23	11.19	7.95
carcass	37.50	44.91	4.22	12.19	4.03	3.73	3.68	2.89
bone marrow	1.67	1.40	0.03	0.06	0.41	0.32	0.24	0.23

In a further experiment, bone marrow and ovary uterus radioactivity at 0.5h, 8h, 24h and 96h was assessed in the females, dosed with a single administation of 50 mg/kg b.w.(Table B.6.1-5c). At the first time-point, slight radioactivity was detectable in the bone marrow (0.09 ppm), and some more in the ovary/uterus (37 ppm). There was a consistent decline over time, such that at termination on d4, the concentration in both tissues was <0.5 ppm.

Table B.6.1-5c : Tissue distribution of radioactivity (50 mg/kg b.w.) as a function of time

h after administration		**0.5**	**8**	**24**	**96**
ovary / uterus	**ppm**	36.88	4.83	0.91	0.48
	%	0.21	0.02	0.00	0.00
bone marrow	**ppm**	0.09	0.06	0.03	0.03
	%	0.00	0.00	0.00	0.00

Tissue concentrations of radioactivity (ring B-^{14}C) expressed either in µg eq/g tissue (ppm) or relative to the administered dose (%).

Only small amounts of remaining radioactivity were found in the liver, organ/tissues, skin and carcass 120 h after dosing, suggesting that kresoxim-methyl does not accumulate (table B.6.1-6).

Table B.6.1-6 : Retention of radioactivity 120 h after dosing.

	remaining radioactivity (% of the administered dose)			
treatment, ^{14}C position, route	**liver**	**organs/tissues**	**skin**	**residual carcass**
1×500mg/kg, ring A,oral	0.01-0.02	<0.005	0.01-0.03	0.06-0.08
1×500mg/kg, ring B,oral	0.01	<0.005-0.01	0.05	0.005-0.76
1×50mg/kg, ring B,oral	0.01	<0.005	0.01	0.5-0.96
1×5mg/kg, ring B, iv	0.005-0.02	<0.005	0.01-0.04	0.4-2.07
14×50mg/kg+1×50mg/kg, ring B, oral	<0.005-0.01	<0.005-0.01	0.01	0.45-0.53

A quantitative whole-body autoradiography was realized in which animals were sacrified at 0.5, 2, 8, 24 and 96 h after a single oral administration.
The results confirm previously reported data ; in both sexes, the fraction of absorbed compound was low and maximum concentration of radioactivity in tissues were obtained at 0.5 and 2 h after dose administration.
The highest levels of radioactivity were associated with the content of the gastro-intestinal tract. Lower concentrations were found in liver and kidney (table B.6.1-7) with trace concentrations in the remaining tissues, namely blood, bone, bone marrow, brain, eyes, Harderian gland, lung, muscle, gonads, salivary glands, skin, thymus and thyroid.
At 96 h post-dose, residual radioactivity was detected only in the GI tract of both sexes and in the skin of the female animals, confirming that the compound does not accumulate.

Table B.6.1-7: Tissues containing the highest level of radioactivity

	maximum concentration of radioactivity (µg eq/g tissue) (ring B-^{14}C)			
sex	**M**		**F**	
time(h)	**0.5**	**2**	**0.5**	**2**
liver	5.269	4.939	4.322	3.854
kidney	6.483	6.514	5.386	5.157

- Metabolism of ^{14}C-BAS 490 F(^{14}C- Reg.n°-242009) in rats.

1.Identification of urinary metabolites:
M2 and M9 were the most important metabolites: they are hydroxylation products of the 490M1, a metabolite obtained as the result of ester cleavage. After oral dosing , M9 was predominant (up to 51% of urine radioactivity) . In urine samples from i.v. dosed female rats, M1(one methyl group less than the parent

molecule) and U20 (eluted with the retention time of the parent) were the most important metabolites (38% and 27% of urine radioactivity, respectively). In urine samples from male rats of this group again M9 was the most predominant (52% of urine radioactivity). The importance of M6 (ring -open structure) and M1 were clearly sex dependent: M6 was more expressed in urine from males ; M1 was markedly higher in all urine samples from female rats. The importance of the other metabolites varied considerably (table 6.5.1-8).

Table B.6.1-8 : Urinary metabolites, in percentage of total radioactivity administered.

	% of the administered dose									
dose (mg/ kg bw)	**5 1× ring B-^{14}C**		**50 1× ring B-^{14}C**		**50 14×unlabelled +1× ringB-^{14}C**		**500 1× ring B-^{14}C**		**500 1× ringA-^{14}C**	
dose group	**A**		**B**		**C**		**D**		**DY**	
route	**iv**		**oral**		**oral**		**oral**		**oral**	
period (h)	0-48		0-24		0-48		0-48		0-48	
sex	M	F	M	F	M	F	M	F	M	F
metabolite identity (M)*										
M1	**3.1**	**24.4**	0.8	**3.9**	0.4	**2.7**	0.2	**2.2**	0.3	**3.8**
M2	**8.4**	**4.9**	**3.1**	**5.5**	**2.0**	**3.4**	**1.5**	**2**	**3.7**	**6.5**
M6	**4**	**1.9**	**3.4**	**0.9**	**2.8**	**1.1**	**1.9**	0.5		
M8		0.1	0.6	0.6	0.1	0.4				
M9	**24.7**	**13.6**	**7.4**	**12.5**	**5.5**	**11**	**2.7**	**4.9**	**7.1**	**15.8**
M11			0.6	0.5	0.1	0.3				
M12									(U40) 0.8	(U40) 1.5
M15				0.5		0.6				
M16			0.3	0.3	0.7	0.2	0.3			
M4+M14 +M20	1.9	0.7	1.8	1.7	1.4	1.6	0.9	1.1	M4:1.2	M4:1.9
M22									0.2	
M24							0.1			
M26	M8,M11,M16,M26: 4.3		0.6	0.4	M26,M11M12: 0.9	0.3	M26,M11M12: 0.8	M8,M11,M12, M16M26:1.4		
(parent)		**U20: 16.3**								
M41									1.1	1.6
M42									0.7	
M37,M43									0.2	
unknown		U17: 2			U3: 0.1	U17A: 0.5		U11: 0.2	U22: 0.1 U22a: 0.1 U35-37: 0.5 U41:0.3	U20: 0.9, U22: 0.2, U22a: 0.2

(M)*: in this and the following tables the metabolic codes were abbreviated to the last characters (i.e.M6 instead of 490M6); former unknow metabolite U40 identified as M12 (Kohl, 1995)

2. Identification of fecal metabolites:
Cold extracts of feces from orally dosed animals contained a predominant metabolite which was identical to unchanged parent compound (51-95% of fecal radioactivity in the 0-24 h fraction). Besides that, only subordinate quantities of the other metabolites were observed. No marked difference could be found between animals which had received label A and label B. Feces extracts from i.v. dosed males showed M2, M9 and M1 as major components but not the parent compound. In contrast, the patterns in feces extracts from i.v. dosed females contained the parent compound as main constituent besides M2, M9 and M1.(table B. 6.1-9)

Table B. 6.1-9: Fecal metabolites, in percentage of total administered radioactivity

	% of the administered dose									
dose (mg/ kg bw) fre-quency	**5 1x(ring B-^{14}C)**		**50 1x(ring B-^{14}C)**		**50 15x(ring B-^{14}C)**		**500 1x(ring B-^{14}C)**		**500 1x (ringA-^{14}C)**	
route	iv		oral		oral		oral		oral	
period	0-48 h		0-24 h		0-48 h		0-48 h		0-72 h	
group	A		B		C		D		DY	
sex	M	F	M	F	M	F	M	F	M	F
metabolite identity										
M0		0.5							2.3	1.3
M1	7.7	2.2	3	2.7	2.1		0.1	7.1	4.4	2.3
M2	8.6	1.3	2.4	1.9	2.7	0.5	0.5	5.8	3.3	3.7
M4	0.4		1.5		1.1	0.5	0.3	2.5	0.9	0.9
M5	0.5							0.1		
M9	9.3	1.5	5.0	4	5.2	6	0.9	13.3	5.9	8.9
M15	0.6		4.1	2.3	1.3	2.7	0.1	3.4		1.6
M24					0.6		0.1			
parent		7.7	35.1	45.7	49.5	47.1	74.9	39.5	51.3	32.6
unknown	f2,f5,f17: 5.6	f2:0.3	f19:1.8		f2:1.3	f2,f3: 0.8	f2,f3,f4,f5,f6: 1.1	f2,f5,f6 f12: 3.4	f5,f17: 3	f5,f6,f17:3.8

3. Identification of biliary metabolites:

Table B.6.1-10 : Biliary metabolites, in percentage of total administered radioactivity after oral administration

% of administered dose (ring B-^{14}C)				
dose(mg/ kg bw)	50 (1×)		500 (1×)	
experimental group	R		S	
period (h)	0-33		0-33	
sex	M	F	M	F
metabolite identity				
M1	5.4	5.2	1.7	1.9
M2, M45	3.3	2.7	1.1	1.2
M9	2.4	2.1	1.1	1.3
M26,M33,M25,M39,M29	14.4	9.7	6.3	3.6
M28, M44	5.0	6.9	0.7	2.9
M31	1.3	3.1	0.5	1.1
M35	2.7	1.8	1.7	0.7
M36,M37,M34	1.4	0.8	0.4	0.2
M44			0.4	0.3

The values of the R-group (low-dose) were amended by the notifier (Kohl, 1998), because there was a wrong assignment of tables to this dose-group.

Aliquots of bile were injected directly into the HPLC to record metabolite pattern.
Based upon the newly submitted data, it appears that biliary excretion is reduced by increasing the administered dose.
Biliary metabolite patterns were rather complex with numerous metabolites, mainly glucuronides (M25,M26, M28, M29,M31, M33, M35 and M 39) , which eluted within a relative small polarity range. The main difference between patterns of male and female rats was the intensity of M28 which was considerably higher in bile from females. (table B.6.1.10). No unchanged parent compound was detected in bile.
M1, M2 and M9 were also recovered in urine.

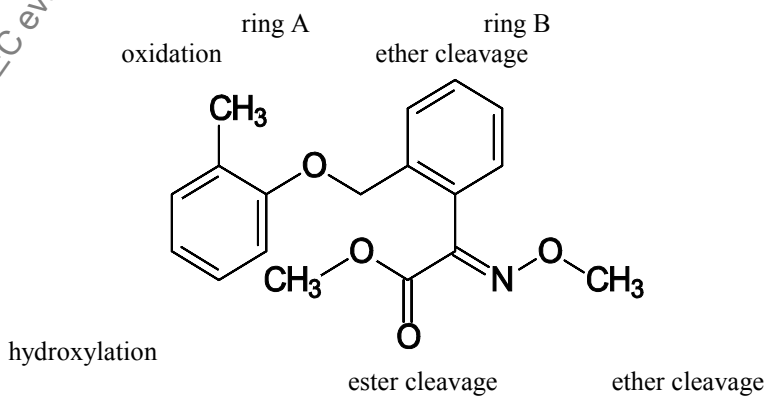
4. Identification of metabolites in plasma, liver and kidney:

As shown in table B.6.1.11, in plasma from both sexes of dose group V, peaks M1, M2 and M9 dominate the metabolite patterns (10-39% of plasma radioactivity). Increasing the dose by a factor of 10 increases generally the plasma, liver and kidney concentration of M1 without affecting the subsequent metabolites M2 and M9 suggesting that hydroxylation but not demethylation is a saturable process. In female M4 and M2 were not completely separated.
M26, a glucuronide deriving from M9 is also important. The remaining peaks were only detected at trace levels.
In plasma from male rats of the dose group W, the metabolite pattern was roughly comparable to the low dose with M1, M9 and M2 as major peaks (7-33% of plasma radioactivity). In plasma from female rats of this group M4 was the second most intense peak (19% of plasma radioactivity) after M1 (47% of plasma radioactivity) followed by M9, M2 and M26.
Liver extracts from male and female rats of dose groups V and W contained M1, M9 and M2 as major peaks (14-43% of liver radioactivity).
Kidney extracts from male and female rats of dose groups V and W contained M1, M9 and M2 as major peaks (12-40% of kidney radioactivity).

Table B.6.1-11: metabolites composition of radioactivity in plasma, liver and kidney of rats after oral administration

	% of tissue activity (ring B-^{14}C)			
dose(experimental group)	50 mg/kg bw (V)		500 mg/kg bw (W)	
period (h)	3.5-4		3.5-4	
	M	F	M	F
metabolites in:				
plasma				
M1	39.4	25	33	46.9
M2	9.7	M2,M4:14	7	10.7
M4	4.3		2.2	18.6
M6	2.7	0.5	0.4	1.6
M9	17.6	13.4	11.1	15.3
M16 M26, M11, M12	12.3		17.2	
M24	3.1	0.2	0.9	0.8
M26		7.2		6.1
unknown		1.9	1.9	0.6
liver				
M1	20.7	24.7	28.6	42.7
M2	13.5	14.5	17.9	13.6
M4	4.1	7.3	4.1	6
M6			2.4	
M9	27.6	27.2	25.6	29.6
M11,M12,M16,M26	13.1	6.7	8.4	4.9
unknown	5.8	2.1		
kidneys				
M1	14.4	17	19.9	31.5
M2	12.3	12.6	17.6	14.1
M4	5.1	4.9	5.2	3.4
M6	5.3	1.4	4.9	1.3
M9	35.5	35.6	38.3	40.2
M16, M26 ,M11, M12:	17.5	6.9	18.3	6.8

Proposed metabolic pathways in rats :



The phase I biotransformation is characterized by six metabolic reactions: (1) cleavage of the ester catalysed by esterases, (2) cleavage of the oxime ether and the (3) benzyl ether bonds; (4) hydroxylation of ring A in para position to the existing oxygen substituent, and (5) oxidation of the aryl-methyl group to the benzyl alcohol and its (6) subsequent oxidation to the corresponding carboxylic acid. The E-Z-isomerisation of the oxime ether group is assumed to be a non-enzymatic reaction catalysed by light and/or acids. The combination of these reactions and the conjugation of the formed OH-groups by glucuronic acid or sulphate leads to the observed large number of metabolites (see Appendix D; p 205)
The metabolite composition in plasma indicates that the **ester cleavage**, which leads to the **free acid 490M1**, is the fasted and **most important detoxification step**.
Mammalian tissues contain a large number of nonspecific esterases that can hydrolyze ester linkages in foreign compounds. The cytosolic esterases are usually associated with a specific reaction, such as acetyl cholinesterase and pseudocholinesterase, whereas the microsomal-associated esterases handle a diverse array of xenobiotic esters. These enzymes can be inhibited when substrates bind tightly to the active sites or when the resulting products are very reactive. This is the case with organophosphates, where metabolites bind to the active site following hydrolysis. Potentiation of anticholinergic effects can be produced by the combined administration of certain pairs of insecticides. The mechanism is explained as an inhibition of the carboxyesterases by one of the two compounds (Ecobichon, 1991, additional information).
A study was undertaken in order to control an eventual potentialization of Kresoxim-methyl toxicity after pretreatment with a cholinesterase inhibitor. In conditions of about 70% erythrocyte cholinesterase inhibition, no toxic effects appeared . These results are explained by the fact that alternate pathways for detoxification of Kresoxim-methyl are involved probably by a direct hydroxylation leading to the production of metabolites 490M24 and 490M15 which were only found in small amounts, since their ester groups are also rapidly cleaved to yield 490M2 and 490M9. Inhibition of ester cleavage, i.e. by organo-phosphate insecticides, would increase hydroxylation to the metabolites 490M24 and 490M15. This pathway seems to be an efficient detoxification pathway as suggested by the results demonstrating that the proximal metabolites 490M24 and 490M15 display a 100 fold reduction in inhibitory activity of the mitochondrial electron transport chain (succinate-dependent reduction of cytochrome-c) compared to the parent compound kresoxim-methyl (additional information , BASF).

Most of the initially formed 490M1 is further metabolised mainly by hydroxylation of the aromatic ring A on the aryl-CH3 group. Thus, after oral administration, the metabolites **490M2**, **490M9** and **their glucuronides** but not 490M1 were the predominant final biotransformation products in excreta. In female rats, these subsequent reactions seem to be quantitatively less pronounced, since females generally excreted higher proportions of 490M1.
Mainly in males the cleavage of the benzyl ether bridge contributes to a certain, but less important extend to the biotransformation of BAS 490F. Fragments containing ring B were mainly recovered in the form of metabolites **490M6** and **490M20**. Ring A is further oxidized to monohydroxybenzyl alcohol, dihydroxybenzyl alcohol and salicylic acid. However, these metabolites as well as o-cresol were only found as conjugates.
The metabolite patterns in excreta of groups receiving a single iv low dose (5mg/kg) and groups receiving a single high oral dose (500mg/kg) were qualitatively and quantitatively comparable. Thus an induction of metabolic
enzymes by the test compound itself can be excluded.

- excretion:

The quantitative urinary or fecal excretion is dependent of the administration route:
After iv administration, 49-66% of the radioactivity was excreted essentially as metabolites via urine and 23-48% via feces (metabolites and parent compound).
After a single oral administration of 50 or 500 mg/kg bw (label B) , 66-67% and 81 % respectively of the administered radioactivity, were excreted via feces in both sexes, while in urine, at low dose, 20-28% and 9-13% at the high dose were excreted. No radioactivity was detected in the exhaled air during the first 48 h. Oral administration leads to the fecal excretion of more unchanged compound than metabolites.
Increasing the dose by a factor of 10 leads to a decreased amount of radioactivity excreted via urine and increased fecal excretion . This indicates that saturation processes (absorption or metabolism) occur. Fecal excretion of unchanged compound increased in male rats while metabolite excretion decreased ; in females,

metabolite excretion was increased or unchanged while excretion of parent compound was not strongly modified.
Repeated administration of the compound did neither change the ratio (urine, feces) nor the time course of the excretion of a single dose of kresoxim-methyl.
With exception of carcass and contents of the GI-tract, radioactivity remaining in tissues after 120 h post-dosing was less than 0.05%. At this time, more than 90% of the administered dose was excreted.
The overall recovery was in the range of 86.4-101.2% in all experiments (table B.6.1.12).
In comparison to the excretion pattern of the A label at the same dose, the amount of radioactivity (label B) excretion via urine was decreased by a factor of 2 - 2.5 in male and female animals respectively.

Table B.6.1-12 : Cumulative excretion in urine and feces after oral or i.v. administration.

sex	route	dose (mg/kg bw) (position of ring label)	period (hr)	mean excretion percentage (cumulative)		
				urine	feces	total + cage wash
M	oral	1x50(B)	0-48 0-120	19.88 20.29	65.31 65.92	86.33
	oral	14x50+1x50(B)	0-48 0-120	14.11 14.59	71.62 73.02	88.29
	oral	1x500(A)	0-48 0-120	16.07 17.28	77.97 80.23	97.33
	oral	1x500(B)	0-48 0-120	8.44 8.68	80.23 80.78	89.65
	i.v.	1x5(B)	0-48 0-120	47.04 49.04	44.72 48.47	101.24
F	oral	1x50(B)	0-48 0-120	27.53 27.94	66.91 67.31	95.57
	oral	14x50+1x50(B)	0-48 0-120	21.82 22.43	66.41 66.86	90.61
	oral	1x500(A)	0-48 0-120	32.25 33.32	59.6 62.06	97.33
	oral	1x500(B)	0-48 0-120	12.90 13.23	81.00 81.3	95.6
	i.v.	1x5(B)	0-48 0-120	64.18 65.87	21.25 22.79	93.24

Table B.6.1-13 : Excretion of radioactivity via bile.

	excretion percentage of radioactivity via bile after a single oral dose (% of administered dose (ring B-^{14}C))			
dose (mg/kg bw)	50		500	
sex	M	F	M	F
maximum excreted (%)	5.43	4.22	1.68	1.13
time(hr)	12-15	15-18	21-24	24-27
cumulative excretion at 48 h (%)	43.1	35.22	14.72	14.01

During the first 48 h after administration, approximatively 35-43% (50mg/kg bw) and 14-15% (500mg/kg bw) were excreted via bile and 19.9-27.5% (50mg/kg bw) and 8.4-12.9%(500mg/kg bw)were excreted via urine. If it is assumed that the amount of radioactivity excreted via bile and urine represents the bioavailable amount of ^{14}C-Reg.n°-242009, then a resorption rate, for the high dose of 23-27% and of 63% for the low dose in both sexes can be calculated.
Increasing in both sexes the dose level by a factor of 10 resulted in an increase of the resorption rate by a factor of 2.5, indicating that the resorption rate is saturated at the high dose level (table B.6.1.13).

Conclusions:
After oral administration , Kresoxim-methyl showed a rapid but saturable and low **absorption** from the GI tract. Radioactivity was mainly excreted via feces (66 to 81% of the dose) essentially as unchanged compound. After parenteral administration the faecal excretion of metabolites and unchanged compound was considerably lower (23-48%) and the proportion found in urine (essentially as metabolites) correspondingly increased. In the original DAR, it was proposed to consider the oral absorption rate 27% at the high dose (500 mg/kg b.w.) and 63% at the low dose (50 mg/kg b.w.), based upon the radioactivity excreted via the urine and the bile. The absorption rate at the low dose is used as a correction factor to establish the AOEL.
Radioactive material was **distributed** in all tissues and organs throughout the body but 96 h after dosing concentration of radioactivity was less than 2% of the administered dose. The highest concentrations of radioactivity were associated with the gastro-intestinal tract and the organs of metabolism and elimination, ie, liver and kidney.
After oral administration of Kresoxim-methyl, the systemically available proportion of compound was rapidly and completely metabolised. The phase I **biotransformation** of the compound in rats comprised the cleavage of the ester, the oxime ether and the benzyl ether bonds, hydroxylation of ring A in para position to the existing oxygen substituent, and it subsequent oxidation to the corresponding carboxylic acid. The resulting OH-groups underwent conjugation with glucuronic acid or sulphate.
Due to saturable absorption **excretion** after oral administration was mainly via feces . After iv application comparable amounts were excreted via urine and feces. There was no evidence of accumulation of radioactive material after repeated dosing.

Metabolism in livestock :

The metabolic pathway of Kresoxim-methyl in the lactating goat was investigated after administration of 454 mg/kg diet for eight days. After isolation from urine, milk and edible tissues , metabolites were identified. Major metabolites were **490M1**(in liver,muscle, minor in milk), **490M2** (liver and kidney), and **490M9** (in muscle, milk and minor in fat). These **major metabolites,** also detected in rats, were formed by **cleavage** of the methyl **ester** bond to the **free acid 490M1**, followed by side chain hydroxylation to 490M2 and aromatic hydroxylation to 490M9. The minor metabolites were **490M6** in fat, liver and kidney ; **490M18** (hypothetical in rats) in all tissues; and **490M19** (not identified in rats; this metabolite is a glycine conjugate) in muscle and liver (see Appendix D; p 205)
Other minor metabolites were 490M56 (not identified in rats), generated by a combination of ring hydroxylation and oxime ether cleavage, and also 490M6, formed by the cleavage of the phenylether bond. Ring hydroxylation without ester cleavage to 490M15 was observed in faeces only. As in milk and tissues, 490M1,

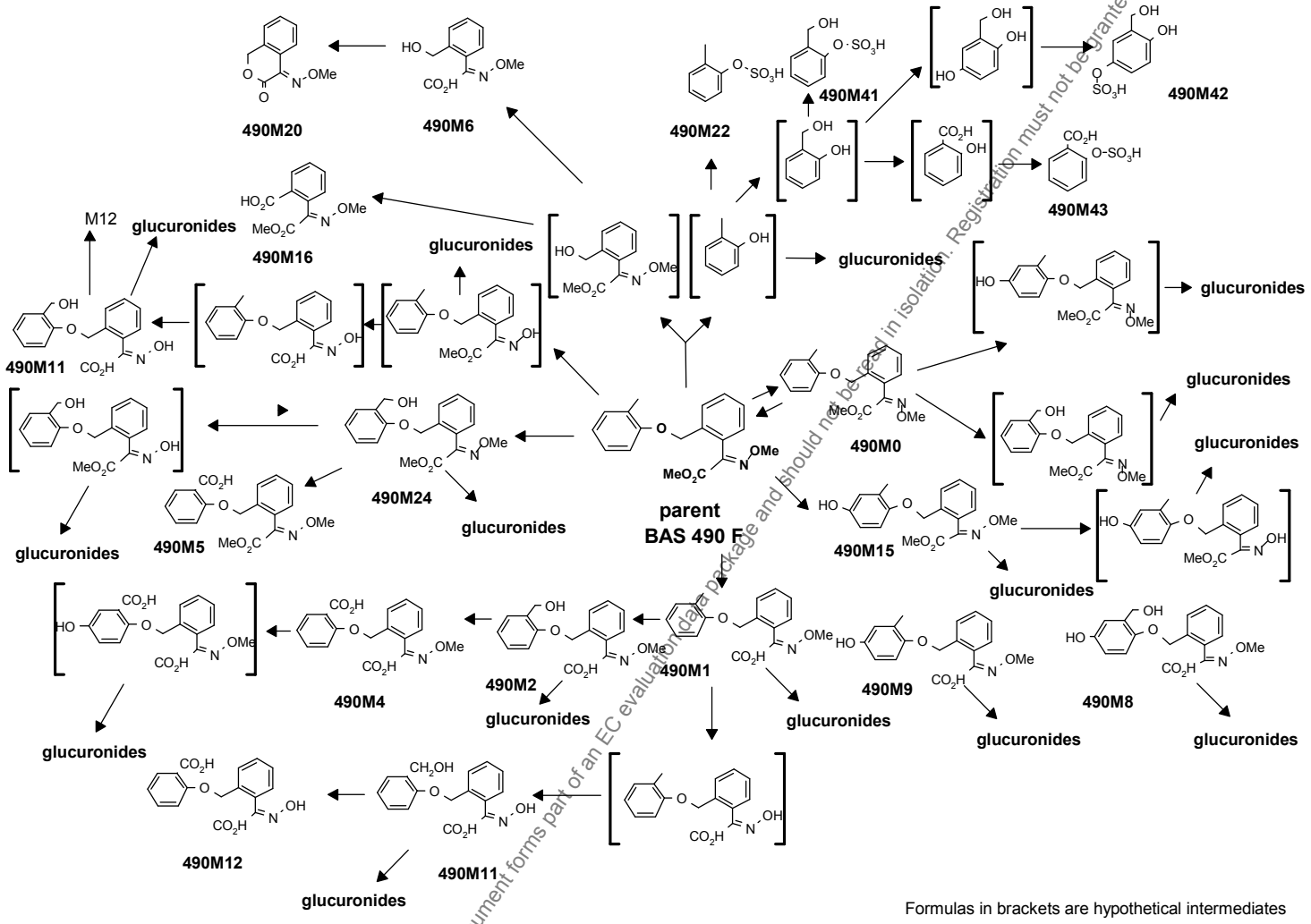
490M2 and 490M9 were major metabolites in urine and faeces. 490M15 was found in faeces only. Minor metabolites in urine and faeces were 490M6, 490M18, 490M19 and 490M56 *(Mayer, 1994).*
The metabolic pathway of BAS 490 F in hens was studied after oral administration of 180 mg/kg food during 6 days. Liver contained as predominant metabolite **490M9** besides the glucuronic acid conjugates **490M28** and **490M63/490M67**. The concentration of the individual metabolites in muscle were extremely low : the main constituent was the sulfate fraction (**490M66/490M51**). Skin and fat contained predominantly nonpolar metabolites (490M58;490M59) and unchanged parent. Negligible radioactivity was recovered in eggs.
The metabolic pathway of ^{14}C-BAS490F is summarised in Appendix D; p 205. The phase I biotransformation is characterized by seven metabolic reactions: **cleavage of the ester** and the benzyl ether bonds; cleavage of the oxime ether bond and oxidation of the oxime to the nitronic acid (this pathway is not observed in rats and leads to the production of 490M59 and 490M58); hydroxylation of ring A in para-position to the existing oxygen substituent, and oxidation of the aryl-methyl group to the benzyl alcohol and its subsequent oxidation to the corresponding carboxylic acid. The combination of these reactions and the conjugation of the formed OH-groups by glucuronic acid or sulfate leads to the observed large number of metabolites *(Grosshans, 1994)*Metabolites 490M58 and 490M59were detected in excreta, muscle and skin. These metabolites represent a minor part of the total residues and are polar.

Metabolism in plants:

In apples, unchanged parent was the predominant radioactive constituent; 490M1 and parent isomer 490M0 were identified. 490M1 give rise to 490M2 and 490M9 which are both conjugate (presumably glucosides) *(Grosshans, 1994a)*. All these metabolites were identified in rats (see Appendix D; p 205).
In wheat, except for the grains, the utmost portion of radioactivity was unchanged parent. In addition,the Z-isomer (490M0) of the parent, the acid 490M1 and conjugates of the alcohol-acid 490M2 and of the phenol-acid 490M9 were identified in straw and forage. In straw an additional metabolite (490M17; 3.6% TRR) was characterized as a ring A hydroxylation product (minor metabolite). The grain extract contained the parent compound *(Grosshans, 1994b)* (see Appendix D; p 205).
In grapes, unchanged BAS 490F or its Z-isomer (490M0) are the predominant radioactive constituents. Three conjugates of hydroxylated metabolites were identified. Smaller amounts of the free acid of BAS 490F (490M1) and the free aglycone of one of the conjugates were also identified (see Appendix D; p 205) (Nelsen et al., 1995).
Samples from a rotational crop study did not contain any unchanged parent or only contained low amounts. Bean and carrot forage and lettuce contained a high percentage of " phase II metabolites", the conjugates of 490M2 and 490M9 *(Grosshans, 1994c)*.

Conclusions:
The major metabolic pathways proposed for the active substance are comparable in rats, goats and hens. However,
an additional minor pathway is observed in hens(cleavage of the oxime ether bond and oxidation of the oxime).
Unchanged parent was the predominant radioactive constituent in plants, but samples from a rotational crop study did not contain any unchanged parent or only contained low amounts and in these cases high percentage of conjugates of 490M2 and 490M9 were identified.

Figure B.6.1: Metabolic pathway of Kresoxim-methyl in rats (next page)



B.6.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

B.6.2.1 Acute oral toxicity (Annex IIA 5.2.1)

- Report : study on the acute oral toxicity of Reg.n°.242 009 in rats (Kirsch et al., 1993, Dossier BASF)
Guidelines:
Protocol in compliance with method B.1 of Directive 92/69/ EEC.
The study is GLP
Material and methods :
5 Wistar (CHBB:THOM,SPF) rats/sex received a single dose of an aqueous suspension of kresoxim-methyl (93.7%;B.n° N36) in tylose CB 30000(0.5%) at a dose level of 5000mg/kg bw.
Findings and conclusion :
No clinical or pathological signs of toxicity have been observed.
LD50 M > 5000 mg/kg bw
LD50 F > 5000 mg/kg bw

B.6.2.2 Acute dermal toxicity (Annex IIA 5.2.2)

-Report: study on the acute dermal toxicity of Reg.n°.242 009 in rats (Kirsch et al., 1993a, Dossier BASF)
Guidelines:
Protocol in compliance with method B.3 of Directive 92/69/EEC.
The study is GLP
Material and methods :
5 Wistar rats (CHbb:THOM(SPF)/sex were exposed to an aqueous suspension of kresoxim-methyl (93.7%;B.n° N36) in a 0.5% solution of tylose CB 30000 at a dose level of 2000mg/kg bw, by dermal semi-occlusive application for 24 h.
Findings and conclusion:
No clinical or pathological signs of systemic toxicity have been observed.
LD50 > 2000 mg/kg bw .
Local effects of dermal irritation such as well defined erythema in 1 male and 1 female, very slight erythema in 3 males and 2 females and very slight oedema in 1 male were reported.

B.6.2.3 Acute inhalation toxicity (Annex IIA 5.2.3)

-Report: study on the acute inhalation toxicity LC_{50} of Reg.n°.242 009 as a dust aerosol in rats, 4-hour exposure (Gamer et al.,1992, Dossier BASF)
Guidelines :
Protocol in compliance with the method B.2 of Directive 92/69/EEC.
The study is GLP
Material and methods:
5 Wistar rats (CHbb:THOM(SPF))/sex were exposed for 4 h by head-nose inhalation exposure to 2.04 and 5.6mg/l of kresoxim-methyl (96.6%;B.n°.N30). The particle size distribution yielded mass median aerodynamic diameters of 1.8 and 2.5 µm which means that a substantial fraction of the dose administered will sedimentate in the small airways.
Findings :
Mortality: no dead occurred
clinical signs:
- High dose:
Male presented a discharged reddish nose (2/5); respiration was sounded (7/10) and irregular (1/10) . Crust formation and bloody nose was observed in 4 male and 2 female .

Eyes were reddish and discharging (5 male and 5 female). Reddish eyelids with crusts were observed in 1 male at each dose.
High stepping gait, reduced general state and squatting posture was observed in 2 male and 1 female. Piloerection was observed in 1 male.
- Low dose:
Male (1/5) presented a discharged reddish nose and eyes.
All animals at both doses, presented an accelerated and intermittent respiration, a fur contamination with urine and attempted to escape.
From day 1 onward no clinical symptoms were detected.
bw determination: no abnormality.
pathology: no pathologic findings.
Conclusion :
LC 50 >5.6mg/l. At this dose clinical signs of systemic toxicity were observed, assuming a ventilation of 7 l /h and a body weight of 333 mg, this means a dose of 475 mg/kg inhaled over 4 hours.

B.6.2.4 Skin irritation (Annex IIA 5.2.4)

-Report: study on the acute dermal irritation/corrosion of Reg.n°.242 009 in rabbit (Rossbacher and Kirsch, 1992, Dossier BASF)
Guidelines:
Protocol in compliance with the method B.4 of Directive 92/69/EEC.
GLP status :
The study is GLP
Material and methods :
2 White Vienna rabbits/sex were exposed to 0.5 gr of kresoxim-methyl (93.7%; B.n°.N36) applied to the intact skin and covered with a semi-occlusive dressing during 4 h.
Findings:
Evaluation of the data, according to the EU methodology, gave the following results:

<Score erythema>$_{24+48+72\ h}$ = 0/0/0
<Score oedema >$_{24+48+72\ h}$ = 0/0/0

Conclusion :
Kresoxim-methyl has no irritant properties and should therefore not be classified.

B.6.2.5 Eye irritation (Annex IIA 5.2.5)

-Report: Study on the acute eye irritation of Reg.n°.242 009 in rabbit (Rossbacher and Kirsch ,1992a, Dossier BASF)
Guidelines, methodology and findings:
Protocol not in compliance with the test method B.5 of Directives 92/69/EEC but in compliance with dir. 84/449/EEC or OECD 405 (1981-87): the lids were not held together for about 1 second to prevent loss of the material. The study is accepted.
GLP status : yes
kresoxim-methyl (93.7%;B.N36) was instillated (0.1 ml ; ±39 mg) into the right conjunctival sac of the eye of 6 New white Vienna rabbits(2M and 4F).
Evaluation of the data, according to the EU methodology, gave the following results:

unwashed eye : <Score cornea opacity.>$_{24+48+72\ h}$ = 0/0/0
<Score iris> $_{24+48+72\ h}$ = 0/0/0
<Score erythema> $_{24+48+72\ h}$ = 0/0/0
<Score chemosis> $_{24+48+72\ h}$ = 0/0/0

Conclusion:
On the basis of this test, kresoxim-methyl has no irritant properties and should not be classified.

B.6.2.6 Skin sensitization (Annex IIA 5.2.6)

-Report on the Maximization test for the sensitizing potential of Reg.n°.242 009 in guinea pigs (Rossbacher and Kirsch, 1993, Dossier BASF)
Guidelines:
Protocol in compliance with method B.6 of Directive 84/449/EEC.
GLP status : yes
Material and methods :
20 female Guinea-pigs (Pirbright White, Dunkin Hartley HOE DHPK(SPF-LAC)BÖ) received an intradermal induction of kresoxim-methyl (93.7%;B.n°.N36) 5% in 0.5% solution of tylose CB 30000 in 0.9% aqueous NaCl solution resp. in Freund's adjuvant/0.9% aqueous NaCl-solution (1:1)resp. 0.5% tylose CB 30000 in 0.9% aqueous NaCl solution.
Percutaneous induction of 50 % test substance in 0.5% Tylose CB30000 in aqua bidest.resp. 0.5% Tylose CB 30000 in aqua bidest. For the challenge reaction, the animals were exposed dermally to 50 % test substance in 0.5% tylose CB 30000 in aqua bidest. resp. 0.5% Tylose CB30000 in aqua bidest., 21 days after the intradermal induction.
A second group with 10 animals served as a control group.
Findings :
Intradermal and percutaneous induction , 5 or 50% resp. , caused slight to well-defined signs of skin irritation and incrustation, partially open in the test group animals. After challenge, no skin reactions were observed either in control or test group animals.
Evaluation of the data, according to the EU methodology, gave :
sensitization rate: 0 % after 48 h (0% for Tylose)
Conclusion :
Kresoxim-methyl should not be classified as a sensitiser.

B.6.2.7 Summary of acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

Table B.6.2.7-1 : Summary of acute toxicity of kresoxim-methyl

Type of test Test species	Result	purity(%)	kresoxim-methyl batch n°.	reference
LD50 oral rat M+F	>5000 mg/kg bw	93.7	N 36	Kirsch et al.,1993
LD50 dermal rat	>2000 mg/kg bw	93.7	N 36	Kirsch et al.,1993a
LC50 rat , 4 h dust M;F	>5.6 mg/l air = (475 mg/kg)	96.6	N 30	Gamer et al.,1992
Skin irritation rabbit 4 h	non irritant	93.7	N 36	Rossbacher and Kirsch,1992
Eye irritation rabbit	not irritant	93.7	N 36	Rossbacher and Kirsch,1992a
Skin sensitization guinea pig M&K	not a sensitizer	93.7	N 36	Rossbacher and Kirsch,1993

Comments: signs of systemic toxicity were seen upon inhalation of a dose of 475 mg/kg over 4 hours, i.e., a dose at least 10 times lower than the sign-free, highest oral dose tested. This is in line with the partial absorption observed in the toxicokinetic studies after oral administration.

B.6.3 Short term toxicity (Annex IIA 5.3)

B.6.3.1 Oral 28 day toxicity (Annex IIA 5.3.1)

- Report: study on the oral toxicity of Reg.n°.242 009 in Wistar rats. Administration in the diet over 4 weeks (range-finding) (Schilling et al.,1992, Dossier BASF)
Supplement: Results and statement of the hormone examinations (TSH, T3, T4) (Wuttke, 1990, Dossier BASF)
Supplement: Pathology report (Bahnemann, 1992, Dossier BASF)
Guidelines:
Protocol not fully in compliance with method B.7 of Directive 92/69/EEC but conform to dir.84/449/EEC.
GLP status : yes
Material and methods :
5 Wistar rats/sex/group were fed a diet containing kresoxim-methyl (96.55%; b.n.: CP 5864) at of 0, 1000, 4000 and 16000 ppm during 4 week.
Converted doses for M : 0; 91; 365; 1428 mg/kg bw/d
F : 0; 95; 375; 1481mg/kg bw/d
Findings :
The main treatment related effects are given in the table B.6.3.1-1 The MTD was reached.

Table B.6.3.1-1 : Oral 28 day toxicity in rats

Endpoint/dose	**0**		**1000 ppm**		**4000 ppm**		**16000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mortality			no treatment related effect					
clinical signs			no treatment related effect					
food consumption						↘		↘
body weight								
bw gain						↘		↘
ophthalmology			no treatment related effect					
hematology			no treatment related effect					
clinical chemistry								
ALT			↘					
AST				↘		↘		
GGT							↗	
albumine							↗	
TSH							↗	↗
urine analysis			no treatment related effect					
organ weight:								
popliteal lymph					↗a,r	↗a,r		
nodes								
liver								
histopathology			no treatment related effect					

statistically significant increase (↗) or decrease (↘) (Kruskal-Wallis test; Mann-Whitney U-test) ; a = absolute; r = relative

Comments:
Food consumption and body weight gain decreased in the last 2 weeks, in females only, but without dose-response relationship suggesting an effect not related to the test substance. The increase in Thyroid Stimulating Hormone (TSH) was not accompanied by changes in T3 or T4 and, being not seen in any of the other tests, this is seen as a chance observation. The same applies to the weight changes in popliteal lymph nodes. The decreases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) could not be clearly explained.
Deviation from the 92/69/EEC protocol: histopathological examination was not performed in all tissues. Heart and thymus were not weighed. Clinical signs not fully described.
Conclusion :
Increased metabolic load and overt signs of toxicity.
NOEL = NOAEL= 4000 ppm = 365mg/kg /d for male and 375mg/kg /d for female rats.
LOAEL = 16000ppm = 1428 mg/kg bw/d in male and 1481mg/kg bw/d in female.

- Additional information supplied by Gelbke, Hildebrand, Jacob and Regenstein, meeting 21 february 1996, Brussels.
Material, methods and findings
Rats received Kresoxim-methyl in their diet during 3 weeks at 0, 200 and 16000 ppm. Hepatic enzyme activities were measured in order to evaluate the inductive activity of the compound.
Total liver cytochrome P450 level was not affected.
Ethoxyresorufin- and pentoxyresorufin- O-deethylase activities which represent respectively CYP IA1 and CYP IIB1, ∑oxidationof lauric acid (which represent CYP IV A) and palmitoyl-CoA-oxidase (peroxisome proliferation) remained unchanged.
Liver glutathione level was unaffected. An important increase (500% of control) in liver GGT activity was observed in male rats exposed at the high dose. This increase paralleled the serum increase observed in the 28 day study .Therefore, kresoxim-methyl is not considered as a microsomal liver enzymes inducer .
CYP 1A1 activity is a sensitive marker of exposure to chemicals that bind to the Ah receptor located in cytosol and /or nucleus and induction reveals therefore cellular Ah receptor interactions. It seems therefore unlikely that kresoxim - methyl would interact with the Ah receptor (Lucier, 1992).

- Report on the study on the oral toxicity of Reg.n°.242 009 in B6C3F1 mice; administration in the diet over 4 weeks (Range-finding study) (Schilling et al.,1992a, Dossier BASF)
Supplement: Pathology report (Bahnemann, 1992a, Dossier BASF)
Guidelines:
Protocol not fully in compliance with method B.7 of Directive 92/69/EEC but conform to dir.84/449/EEC.
GLP status : yes
Material and methods :
5 B6C3F1/ CrIBR mice/sex/group were fed a diet containing kresoxim-methyl (96.55%; b.n°: CP 5864) at 0, 500, 2000 and 8000 ppm during 4 weeks.(Converted doses : 0, 113, 485 and 2141 mg/kg bw/d in males
0, 182, 798 and 3755 mg/kg bw/d in females)
Findings :
There were no treatment related effects on mortality, clinical signs, food consumption, bw gain, ophthalmology, hematology, urine analysis and macroscopic and histological findings. A statistically significant decrease was observed in the highest dose males in number of lymphocytes, cholesterol and triglycerides in serum. In the highest dose group relative liver weight was increased in males and females, in females a relative increase in brain weight was also observed.

Deviation from the 92/69/EEC protocol: histopathological examination was not performed in all tissues. Heart was not weighed. Clinical signs not fully described.
Conclusion
Target organ: the liver, without overt signs of toxicity.
NOAEL = NOEL = 2000 ppm = 485 mg/kg bw/d for males and 798 mg/kg bw/d for females.
LOAEL = 8000 ppm = 2141 mg/kg bw/d for males and 3755 mg/kg bw/d for females

B.6.3.2.1 Oral 90 day toxicity (rat) (Annex IIA 5.3.2)

-Report: subchronic toxicity study with Reg.242 009 in Wistar rats. Administration in the diet over 3 months.
(Mellert et al.,1994, Dossier BASF)
Supplement: Pathology report (Bahnemann, 1994, Dossier BASF)
Guidelines:
Protocol in compliance with the method B of Directive 87/302/EEC.
The study is GLP
Material and methods :
5 groups of 10 Wistar rats (Chbb:THOM (SPF))/sex were fed a diet containing Reg.242 009 (98.7 %, B.n°. N21) at concentrations of 0, 500, 2000, 8000 and 16000 ppm over a period of 3 months.
Mean daily test substance intake: 0, 36, 146, 577 and 1170 mg/kg/d for males
0, 43, 172, 672 and 1374 mg/kg/d for females

Findings :
The main treatment related effects are given in the table B6.3.2.1-1. The MTD was reached.
Table B.6.3.2.1-1 : Oral 90 day toxicity in rats .

endpoint/dose	**0**		**500 ppm**		**2000 ppm**		**8000 ppm**		**16000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mortality			no substance related effects							
clinical signs			no substance related effects							
food consumption			no substance related effects							
body weight							↘9%		↘6%	
body weight gain							↘14%		↘9%	
ophthalmology			no substance related effects							
hematology			no substance related effects							
clinical chemistry:										
AP					-	↘	↘	↘	↘	↘
GGT							↗		↗	
urine analysis			no substance related effects							
organ weight :										
liver			↗r					↗r	↗r	↗r
histopathology:										
fat deposits liver								↘	↘	↘

Statistically significant increase (↗) or decrease (↘) ($p<0.05$, ANOVA or Dunnett's test) r = relative

Other findings:
Transient decreases in ALT and AST were observed in both males and females from the dose of 2000 ppm on.
Comments:
Decreases in alkaline phosphatase (AP) are commonly seen with decreased food intake, which was not obvious here. Similarly, decreases in ALT and AST and decreases in fatty deposition in the liver are not easy to explain, but are probably of less toxicological significance. Nevertheless, together with the relative increase in liver

weight and the increase in gamma glutamyltranferase (GGT) they point to the liver as target organ, but without overt signs of toxicity.

Conclusion :
target organ: the liver, signs of metabolic overload without overt hepatotoxicity.

NOEL =500 ppm= 43 mg/kg bw for females (based upon slightly decreased AP levels at the next dose)
NOAEL =2000 ppm = 146 mg/kg bw for males and 172 mg/kg bw/d for females,
based upon significantly increased GGT levels, increased relative liver weights and decreased body weight (gain) at LOAEL = 8000 ppm and above.

B.6.3.2.1 Oral 90 day toxicity (mice)

-Report: subchronic toxicity study with Reg.242 009 in C57 BL mice. Administration in the diet for 3 months.
(Mellert et al.,1994a, Dossier BASF)
Supplement: Pathology report (Bahnemann, 1994a, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the method B of Directive 87/302/EEC.
The study is GLP
Material and methods :
5 groups of 10 C57 BL/6N CrlBR mice/sex were fed a diet containing 0, 250, 1000 , 4000 and 8000 ppm kresoxim-methyl (98.7 %, B.n°. N21) over a period of 3 months.
Mean daily test substance intake: 0, 57, 230, 909 and 1937mg/kg/d for male
0, 80, 326, 1326 and 2583 mg/kg/d for female
Findings :
There were no treatment related effects on mortality, clinical signs, food consumption, food efficiency, hematology, clinical chemistry and histopathology. The terminal body weight was decreased in male animals treated with the high dose. The relative liver weight was increased in male mice after treatment with 4000 and 8000 ppm. Females were not affected.
Deviation from the official protocol: ophthalmoscopy was not performed.
Conclusion:
The target organ: the liver, without overt signs of toxicity.
NOAEL = NOEL=1000 ppm=230 mg/kg bw for male and 2583 mg/kg bw (8000 ppm) for females.
LOAEL = 4000 ppm = 909 mg/kg bw/d for males.

B.6.3.2.2 Oral 90 day toxicity (dog)

-Report: subchronic toxicity study with Reg.242 009 in Beagle dogs. Administration via the diet over 3 months.
(Mellert et al.,1994b, Dossier BASF)
Supplement: Pathology report (Bahnemann, 1994b, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the method B of Directive 87/302/EEC or OECD 408(1981).
The study is GLP
Material and methods :
4 groups of 6 pure bred Beagle dogs/sex were fed a diet containing kresoxim-methyl(B.n°. N27; 94%; N30 : 96.6%) at concentrations of 0, 1000, 5000 and 25000 ppm over a period of 3 months.
Mean daily test substance intake: 0, 30, 150, 776 mg/kg/d for males.
0, 34, 168, 846 mg/kg bw/d for females
Findings :
There were no treatment related effects on mortality, clinical signs, food consumption, food efficiency, hematology, clinical chemistry and histopathology.
Transient decreases in body weight were observed. At the highest dose, vomiting and diarrhea were observed. Food consumption decreased in females in the control group and in the two highest dose groups. This brought about a decrease in bw gain in females of the highest dose group. As far as clinical chemistry is concerned, a

transient decrease in albumin and total protein concentrations was probably the result of vomiting and diarrhea. Serum albumin concentration remained lowered in the 5000 ppm group females.
Beside a clear impact on the gastrointestinal system, kresoxim-methyl did not produce relevant systemic toxicity in the dog.

Deviation from the official protocol: blood samples were taken on day 32, 88 and 86 after the beginning of the administration period and not at the beginning, then either at monthly intervals or midway through the test period, and finally at the end of the test period.
Conclusion:
Target organ : gastro-intestinal irritation.
NOEL = 1000 ppm ; NOAEL=5000ppm = 150 mg/kg bw for males and 168 mg/kg bw for females, neglecting the small decrease in serum albumin.
LOAEL = 25000 ppm = 776 mg/kg bw/d for males and 846 mg/kg bw/d for females.

B.6.3.2.3 Oral 1 year toxicity (dog) (Annex IIA 5.3.2)

- Report on the study of the toxicity of Reg.n°.242 009 in Beagle dogs. Administration via the diet over 12 months.(Hellwig et al.,1994, Dossier BASF)
Supplement: Pathology report (Bahnemann, 1994c, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the method B of Directive 87/302/EEC but in compliance with OECD 452 guideline(1981).
GLPstatus : yes
Material and methods :
4 groups of 5 pure bred Beagle dogs/sex were fed a diet containing kresoxim-methyl (B.n°. N36, 93.7%) at concentrations of 0, 1000, 5000and 25000 ppm over a period of 12 months.
Mean daily test substance intake: 0, 27, 138, 714mg/kg bw/d in male
0, 30, 146, 761 mg/kg bw/d in female.
Deviation from the official protocol: at the beginning of the study the weight variation in the animals used exceeded ± 20% of the mean value.
Findings :
The main treatment related effects are given in the table B.6.3.2.3.1. The MTD was reached.

Table B.6.3.2.3-1: Oral 1 year toxicity in dogs.

endpoint/dose	0		1000 ppm		5000 ppm		25000 ppm	
	M	F	M	F	M	F	M	F
mortality								
clinical signs			no treatment related effects					
food consumption		↘		↘		↘		↘
food efficiency							↘	
body weight							↘(11%)	
ophthalmoscopy			no treatment related effects					
hematology								
thrombocytes			↗		↗		↗	
clinical chemistry			no treatment related effects					

urine analysis			no treatment related effects
organ weight			no treatment related effects
histopathology			no treatment related effects

Statistically significant increase (↗) or decrease (↘) (p<0.05) ; Kruskal-Wallis-h-test; Mann-Whitney-U-test

Comments:
The increase in platelet number is dose-dependent, however, all the numbers found were within the historical control limits for this sex and strain of dogs. There were no other effects on blood coagulation.
Besides an effect on food intake, no clear signs of toxicity were observed in this experiment. This is in agreement with the 90 day dog oral study.
Deviation from the official protocol: the highest dose level did not elicit definite signs of toxicity. This dose was selected according to the current MTD concept of U.S.-EPA.
Conclusion:
NOEL < 1000 ppm ;
NOAEL=5000 ppm=138 mg/kg bw/d for males and 25000 ppm = 761 mg/kg bw/d for females, not taking into account the small effect on food intake and increase in number of platelets.
LOAEL = 25000 ppm = 714 mg/kg bw/d.

B.6.3.2.4 Oral 6 months toxicity (rat)

no data, not necessary.

B.6.3.2.5 Additional information related to the oral subchronic observations.

Effect on the liver mitochondria in rats .

- Report: Reg.n°.242009- Electron microscopic examinations of liver samples to assess mitochondria from old Wistar rats treated for 3 weeks in the diet (Mellert et al., 1995a) (Dossier BASF)
Material, methods and findings:
3 female Wistar (Chbb: Thom (SPF)) rats /dose at an age of about 15 months received in the diet kresoxim-methyl (N36, 94.3%) at 0, 200 and 16 000 ppm for 3 weeks. Light and electron microscopy of liver samples was initiated to control if there were any substance-dependent structural or quantitative changes on hepatocyte mitochondria of old female rats.
Literature data suggest that cristae mitonchondriales of normal liver cells are known to be sparse. Liver cells contain mitochondria with a predominance of matrix enzymes because of the metabolic activity of hepatocytes in contrast to mitochondria of high energy consuming organs. An increase or decrease in the amount of cristae is known to be a drug response to toxic effects. Dense granules are known to disappear in cases of a pathological (degenerative) swelling of mitochondria, and an increase or decrease is described in literature as drug response toxic effect.
In this study, dense granules are present in mitochondria of control and high dose animals. They are observed in similar proportions, as described in literature for normal cells. No alteration could be noted. Neither the structure, nor the amount of mitochondria were seen to be altered, when compared with the mitochondria of the control hepatocytes.
The study is GLP.

Effect on liver peroxisome proliferation in rats.

- Report: Reg.n°.242009- Electron microscopic examinations of liver samples to assess peroxisomes from Wistar rats treated for 3 weeks in the diet. (Mellert et al., 1995b) (Dossier BASF)
Material, methods and findings:

3 female Wistar (Chbb: Thom (SPF)) rats /dose received in the diet kresoxim-methyl (N36, 94.3%) at 0, 200 and 16 000 ppm for 3 weeks. Light and electron microscopy of liver samples was initiated to control if there are any substance-dependent structural or quantitative changes on peroxisomes observed after administration of the compound to female rats.
Peroxisomes contain a variety of H_2O_2-producing oxidases and a large quantity of enzyme catalase which detoxifies accumulating H_2O_2. They are the exclusive site for oxidation of the very long chain fatty acids. The cytochemical detection of catalase using 3,3'-diaminobenzidine as oxidizing agent helps to visualize hepatocyte peroxisomes in general and alterations in shape, size and number in particular. No alterations (especially proliferation) could be observed in this study after treatment with kresoxim-methyl, neither by light microscopy of all test animals, nor by a thorough electron microscopic investigation of selected liver samples of control and high dose animals.
The study is GLP.

Investigation on the origin of reduced enzyme levels in rats after adminsitration of kresoxim-methyl.

- Effects of Reg.n°.242 009 on enzyme levels in rat serum (Moss, 1994, Dossier BASF)

Material, methods and findings:

First study: 5 Wistar rats/sex received in their diet 8000 ppm of kresoxim-methyl for 2 weeks. Blood was sampled before exposure and after treatment. Sera were analysed for total alkaline phosphatase activity and isoenzyme composition. Liver and small intestine were collected from 2 animals to prepare tissue extracts.
Second study: animals were exposed to 8000 ppm of kresoxim-methyl for 1 week via the diet and the effects of dietary manipulation (diet supplemented with olive oil or sucrose) on changes in serum enzyme activities in female rats were examined.

Total serum AP activity in treated animals fell to about 66% of the pre-treatment levels.
Intestinal phosphatase was lower in sera from treated than from untreated rats, indicating that the fall in total alkaline phosphatase activity was due to a reduction in the contribution of the intestinal isoenzyme.
2. In rats, serum AP activity increases after ingestion and intestinal absorption of fats.
Addition of fat to the diet of untreated rats produced, as expected, a marked increase in total AP activity due mainly to an increase in the contribution of the intestinal isoenzyme without contribution of the liver/bone alkaline phosphatase activity . Administration of kresoxim-methyl had no effect on the liver/bone alkaline phosphatase activities of animals receiving the olive oil supplement but a fall in total and intestinal activities in serum occurred.
The relationship between dietary status and serum AP was also observed in fasted animals in which this activity was markedly lower than in fed animals and affected the intestinal enzyme. kresoxim-methyl administered to fasted rats produced a small insignificant fall in the serum residual activity.
3. Addition of sucrose to the diet: the total serum AP activity of untreated fasted rats was lower than that of animals receiving a normal diet, because of the considerable reduction of contribution of the intestinal isoenzyme. Levels of liver/bone isoenzyme activity were of the order of those of untreated animals receiving a normal or olive oil enriched diet. In sera of animals receiving a sucrose enriched diet, total AP and intestinal activity were not significantly affected by the administration of kresoxim-methyl.
Reduction of intestinal AP in serum was not due to inhibitors in the serum (metabolites or kresoxim-methyl).
Treatment with kresoxim-methyl did not cause a reduction in the intestinal AP content of the tissue.
4. No evidence was found of any interference by kresoxim-methyl or its metabolites with the reaction of ALT with its cofactor. Dietary supplementation with either extra fat or carbohydrate appears to prevent the fall on treatment with kresoxim-methyl seen, though inconsistently, in animals on a unsupplemented diet. Thus, reductions in serum ALT activity on treatment with kresoxim-methyl, when they occur, also seem to be related to alimentation, though neither the degree of change in ALT nor its relation to diet is as obvious as is the case for intestinal AP and fat intake.

Conclusion:

In rats, fall of total serum AP activity is due to a reduction of the contribution of the intestinal isoenzyme which appears to be sensitive to dietary manipulations.
In man, little or no intestinal AP is demonstrable in plasma, even after ingestion of fat, because the isoenzyme is rapidly cleared from the circulation through uptake by receptors on hepatocytes. Low or absent plasma intestinal AP has no significance in human chemical pathology.

- Report: test study on enzyme activity after treatment with Reg.n°.242009 in Wistar rats. Dietary administration for 3 weeks and recovery of 2 weeks (Mellert et al.,1995, Dossier BASF)
Material, methods and findings:
10-15 Wistar (Chbb: Thom (SPF)) rats /sex/dose received in the diet kresoxim-methyl (N30, 96.6%) at 0, 10, 50, and 8000 ppm for 3 weeks; 10 animals /sex were sacrificed after 3 weeks of treatment. The remaining 5 rats of control and high dose group were maintained for another 14 days without administration of compound.
At 8000 ppm: serum ALT (significantly) and AP (trend towards reduced activity) were decreased already 24 h after beginning of treatment without further reduction in the course of the study. These effects were reversible after withdrawal of the test compound for 8 days. In males, GGT(statistically significant) in liver homogenate was increased after 23 days of test substance intake and returned to normal values at the end of a 2 week recovery. This effect is considered to be treatment -related and indicative of hepatocellular damage.
At 50 and 10 ppm : no treatment -related changes in ALT and AP .
The NOEL of this study is 50 ppm (5 mg/kg bw/d).
The study is GLP.

B.6.3.3.1 28-day inhalation toxicity (rat)
No data, not necessary.

B.6.3.3.2 90-day inhalation toxicity (rat)
No data, not necessary.

B.6.3.3.3 28-day dermal toxicity (rat)

- Report : Study of the dermal toxicity of Reg.242 009 in Wistar rats. Application to the intact skin over 3 weeks. (Kirsch et al.,1994, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the method B.9 of Directives 92/69/EEC (or 84/449/EEC) but conform to the OECD 410 guideline(1981).
GLP status : yes
Material and methods :
Kresoxim-methyl (94.3%; b.n°: N 36) was applied daily for 6 h to the clipped intact dorsal skin under semi-occluded patch of 5 Wistar (Chbb=THOM) rats/sex over a period of 3 weeks at a dose of 1000mg/kg bw. A control group of 5 rats/sex was exposed to the solvent (Tylose CB 30000).
Deviation from the official protocol: the exposure period of 21 d is too short (28 d).This deviation does not compromise the significance of the results.
Findings :
There were no treatment related effects on mortality, clinical signs, food consumption, body weight gain, hematology, clinical chemistry , urine analysis and organ weight. In male rats a lower fat deposition in the liver was observed, similar to the 90 day oral study, the significance of which is not clear.
A local effect, a minute superficial crust in the outer layer of the cornified epithelium, was observed in 1 male rat but did not seem toxicologically relevant.
Conclusion:
The NOAEL=NOEL=1000 mg/kg bw.

B.6.3.3.4 90-day dermal toxicity (rat)
No data, not necessary

B.6.3.4 Summary of short-term toxicity (Annex IIA 5.3)

After subchronic oral exposure at the MTD of kresoxim-methyl, rats showed increases in liver weight, serum albumin and GGT, decreases in the number and the content of fat containing vacuoles in the liver, decreases in

serum AP, ALT and AST, without overt signs of hepatotoxicity. At the 28-day MTD dose no alterations could be observed at the electron microscopic level in the liver peroxisomes or mitochondria. Although no clearcut changes occurred in food intake, which would have explained a decrease in serum AP, additional information suggests that this might be due to a slight interference with fat absorption. Decreases in serum ALT and AST remain more difficult to explain, but being accompanied by an increase in GGT, suggest some metabolic stress. An increase in TSH levels, without changes in T3 or T4 and without enzyme induction, an isolated finding in the 28-day rat test, is interpreted as a chance finding.

Negative results were obtained in a short-term interaction study performed to assess whether inhibition of esterases that detoxify Kresoxim-methyl by cholinesterase inhibitors may potentiate the toxicity of Kresoxim-methyl.

Mice responded in a similar way, although the changes were less pronounced.

Dogs exhibit altered food intake, and reacted with vomiting and diarrhea, with impact on body weight and transient decreases in serum albumin and protein concentrations but without signs of organ toxicity.

Table B.6.3.4-1 : Summary of short-term toxicity of kresoxim-methyl

Type of test Test organism	**Results**			**kresoxim-methyl, purity (%) batch number**	**References**
	NOAEL mg/kg bw/d	**LOAEL mg/kg bw/d**	**critical effects**		
28 d , p.o., rat	M: 365 F: 375	1428 1481	↗ serum GGT, albumin, TSH	96.5 % CP 5864	Schilling et al., 1992
28 d, p.o., mice	M: 485 F: 798	2141 3755	↘ triglycerides, cho-lesterol and lymphocytes	96.5 % CP 5864	Schilling et al., 1992a
90 d, p.o., rat	M: 146 F: 172	577 672	↗ GGT, ↗relative liver weight, ↘body weight (gain)	98.7 % N 21	Mellert et al.,1994
90 d, p.o., mice	M: 230 F: 2583	909 -	altered weight parameters	98.7 % N 21	Mellert et al.,1994a
90 d, p.o., dog	M:150 F: 168	776 846	↘ serum albumin and protein; ↘ body weight gain	94%, N 27; 96.6%, N 30	Mellert et al.,1994b
21 d, dermal, rat	1000	-	no effect	94.3% N 36	Kirsch et al.,1994
1 yr, p.o. ,dog	M: 138 F : 761	714 -	↘ body weight	93.7 % N 36	Hellwig et al.,1994

B.6.4 Genotoxicity (Annex II A.5.4.)

B.6.4.1 *In vitro* genotoxicity testing (Annex IIA 5.4.1)

B.6.4.1.1 Gene mutation test in bacterial cells

- Report on the study of Reg.n°42 009 in the Ames Salmonella/mammalian -microsome mutagenicity test and Escherichia coli/mammalian-microsome reverse mutation assay (Standard plate test and preincubation test) (Gelbke et al., 1993, Dossier BASF)

Guidelines:
The protocol of the study with S.typhimurium is not fully in compliance with the method B 14 of Directive 92/69/EEC.
GLP status : yes
Material and methods :
Salmonella strains TA 98, TA 100, TA 1535, and TA 1537 were used. E.coli WP2uvrA was included in the test. The standard plate incorporation assay as well as the preincubation test in liquid medium were realized using 20, 100, 500, 2500 and 5000 µg/plate of kresoxim-methyl (93.7%; B.n° : N 27) with and without S9mix (S9 conc.?) (Aroclor 1254 induced rat liver). Each experimental point was performed in triplicate .
The solvent used was DMSO. A negative control was realized with the solvent. Both studies were performed in good experimental conditions; the criterias for the determination of a positive response were well defined and appropriate.
Positive controls +S9: 2-aminoanthracene
Positive controls - S9: N-Methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine and N-ethyl-N'-nitro-N-nitroso-guanidine.
Positive controls gave the expected response.
Findings :
No significant increase in the number of revertants, either in the presence or absence of a metabolic activation system is reported. A precipitation of the test substance was found from about 500µg/plate onward.
No bacteriotoxicity was observed.
Minor deviation from the official protocol: the results of untreated controls are not reported.
Conclusion :
Kresoxim-methyl is not mutagenic in these experimental conditions.

- Report on the study of Reg.n°242 009 in the Ames test (Salmonella/mammalian -microsome mutagenicity test - Standard plate test and preincubation test) (Gelbke et al., 1994, Dossier BASF)

Guidelines: Protocol of the assay performed with S.typhimurium not fully in compliance with the method B.14 of dir. 92/69/EEC .
GLP status : yes
Material and methods :
Salmonella strains TA 98, TA 100, TA 1535, and TA 1537 were used. The standard plate incorporation assay as well as the preincubation test in liquid medium were realized using 20, 100, 500, 2500 and 5000 µg/plate of kresoxim-methyl (94.3%; B.n° : N36) with and without S9mix (S9 conc.?) (Aroclor 1254 induced rat liver). Each experimental point was performed in triplicate. The solvent used was DMSO. A negative control was realized with the solvent.
Positive controls +S9: 2-aminoanthracene
Positive controls - S9: N-Methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, and 9-aminoacridine.
Positive controls gave the expected response.
The study is performed in good experimental conditions; the criterias for the determination of a positive response are well defined and appropriate.
Findings : No significant increase in the number of revertants was observed either in the presence or absence of a metabolic activation system . Test substance precipitation(2500 µg/plate) and cytotoxicity (500 µg/plate) occurred for strains TA 100 and TA 1537 . Test substance precipitation was observed at 2500 µg/plate for strains TA 98 and TA 1535.

Cytotoxicity occurred at doses >2500 µg/plate.
Minor deviation from the official protocol: the results of untreated controls are not reported.
Conclusion *:*
Kresoxim-methyl is devoid of mutagenic potential under the conditions of this test.

B.6.4.1.2 Gene mutation test in mammalian cells

- Report: gene mutation test in chinese hamster ovary cells (HPRT locus assay) with Reg.n°.242 009 (Pôlloth and Hoffmann,1994, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the method B of dir. 87/302/EEC .
GLP status : yes
Material and methods *:*
Two independent experiments were carried out with CHO-K1 cell line, both in the absence and in the presence of S9 mix (liver S9 from Aroclor 1254 pretreated rats; conc.of S9 used ?), using identical procedures. Ca 500,000 cells/flask were exposed during 4 h to kresoxim-methyl(94.3%;B.n°:N.36) solubilized in DMSO at 0; 0.0001; 0.0005; 0.001; 0.005; 0.01; 0.05 and 0.1 mg/ml (exp.1) or at 0; 0.001; 0.00215; 0.00464; 0.01; 0.0215; 0.0464 and 0.1 mg/ml (exp.2). At the end of 1 week expression period, cells (6x 300000 cells) were seeded to determine the number of mutant colonies (selection period of 1 week); cytotoxicity was determined in parallel. In parallel to the selection of mutants the cloning efficiencies were determined.
Untreated controls were realized with culture medium. Solvent controls were realized with DMSO and culture medium.
Positive controls - S9 : ethylmethanesulfonate
Positive controls + S9 : 3-methylcholantrene
Positive controls gave the expected response.
The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate.
Findings *:*
Both with and without metabolic activation no statistically significant increases in mutant frequencies were observed. No evidence of a dose response relationship was found in any of the experiments.
Precipitation of the test substance was observed at concentrations >0.0215 mg/ml.
Minor deviation from the protocol: mean and standard deviation are not calculated.
Conclusion*:*
Kresoxim-methyl is not mutagenic under the test conditions employed in this *in vitro* test system.

B.6.4.1.3 *In vitro* chromosome aberration assay on human lymphocytes

- *In vitro* cytogenetic investigations of Reg.n°.242 009 in human lymphocytes (Engelhardt and Hoffmann, 1993, Dossier BASF)
Guidelines :
Protocol not fully in compliance with method B10 of dir. 92/69/EEC.
GLP status: yes
Material and methods *:*
Heparinized human venous blood was used; kresoxim-methyl (98.7 %, B.n° : N 21) solubilized in acetone was added to the blood culture at the following concentrations: 0, 2.5, 5, 10, 20 and 40 µg/ml. Test was performed with and without S9 (Aroclor 1254 induced rat liver ; conc.of S9 used ?). After about 3 h of incubation at 37°C, cells were washed and then re-incubated in complete medium for further 21 h. The whole duration of incubation was 72h.
Positive controls -S9: mitomycin
Positive controls +S9: cyclophosphamide.
Negative controls: untreated and acetone .
Positive controls gave the expected response.
Deviations from the official protocol: limited number of metaphases analysed for the positive control (50 instead of 100). Exposure time of 3 h does not cover the length of a whole cell cycle; if for toxicity reasons this

treatment does not cover the length of a whole cell cycle, multiple fixation times are to be chosen. Low tested doses (limited solubility of the compound; conform to OECD 473,1983).
Findings :
None of the tested concentrations induced a statistically and biologically significant increase in the number of aberrant metaphases neither in the presence nor in the absence of S9. The highest experimental dose (40 µg/ml) which led to a compound precipitation did not reduce the mitotic activity .
Conclusion *:*
Kresoxim-methyl is not clastogenic in human lymphocytes under the conditions of the test.

B.6.4.1.4 *In vitro* unscheduled DNA synthesis in mammalian cells .

- *In vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes with Reg.n°.242 009 (Pôlloth and Hoffmann, 1994a, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the test method of annex V (dir. 87/302/EEC).
GLP status : yes
Material and methods *:*
Freshly isolated rat hepatocytes (Wistar; Chbb=THOM (SPF))were used in 2 independent experiments. Approx.400,000 viable cells/well were exposed during 18 hrs to kresoxim-methyl (94.3%;B.n°. N 36) solubilised in DMSO at 0.33; 1; 3.3; 10; 33 and 100 µg/ml.
Cytotoxicity was assessed by measuring the extra-cellular lactate dehydrogenase activity. UDS was quantificated by autoradiography.
2-AAF used as positive control, gave the expected response.
The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate.
Deviation from the official protocol: the amount of ^{3}H-TdR incorporation in the cytoplasm should be determined by counting three nucleus-sized areas in the cytoplasm of each cell counted and not in the most heavily labelled nuclear sized area of the cytoplasm.
Findings and conclusion :
The repair activity was not induced as compared to the solvent control and no dose-related trend was evident. A marked cytotoxicity was observed at concentrations >10 µg/ml.

B.6.4.2 *In vivo* genotoxicity testing (somatic cells) (Annex IIA 5.4.2)

B.6.4.2.1 *In vivo* mammalian bone-marrow micronucleus test.

B.6.4.2.1a *In vivo* mouse bone-marrow micronucleus test.

-Report: cytogenetic study *in vivo* of Reg.n°.242 009 in mice micronucleus test. Single intraperitoneal administration. (Engelhardt and Hoffmann, 1993a, Dossier BASF)

Guidelines:
Protocol in compliance with the method B.12 of Directive 92/69/EEC.
GLP status : yes
Material and methods *:*
5 NMRI (Charles River) mice/sex were treated with a single intraperitoneal administration of kresoxim-methyl (93.7%; N 36) suspended in CMC (0.5%) , at 500, 1000 and 2000 mg/kg body weight. Animals were sacrificed at 16, 24, 48 hrs in the highest dose group and after 24h for positive controls , solvent control and low doses.
Bone marrow from femur were prepared.
Negative control (vehicle): CMC (0.5%CMC).
Positive controls : cyclophosphamide or vincristine.
The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate. Positive controls gave the expected response.
Findings *:*

No increase in the rate of micronuclei was detected at any of the sampling times. No inhibition of erythropoiesis was detected.
Conclusion :
Kresoxim-methyl, at toxic doses , did not show chromosome breaking or spindle poisoning activity resulting in the formation of micronuclei under the experimental conditions.

B.6.4.2.1b *In vivo* rat bone-marrow micronucleus test.

-Kresoxim-methyl, oral (gavage), rat bone marrow, micronucleus *in-vivo*, 500- 2000 mg/kg b.w. (Engelhardt, 1997)

Findings:
No clinical signs were observed at any dose, and the ratio PCE/NCE was unaltered in all animals treated with Kresoxim-Methyl, as compared to the controls.
The incidence of micronucleated PCE was unaltered by the treatment with Kresoxim-Methyl, whereas the positive control induced the expected number of micronuclei at both sampling times. The observed MN were of the small type (<1/4 of the nucleus diameter). The treatment was also without effects on the number of MN within NCE's (latter data not tabulated).

Table B.6.4.2.1-1: micronucleus incidence in rat bone marrow cells treated with Kresoxim-Methyl

		♂			♀		
Sampling time (h)	**Dose (mg/kg bw)**	**n**	**‰ MNPC**	**PCE/NCE**	**n**	**‰ MNPC**	**PCE/NCE**
24	0	5	2.4	1.29	5	2.1	1.34
	500	5	1.4	1.18	5	1.6	1.19
	1000	4[1]	2.0	1.40	5	2.0	1.24
	2000	5	1.6	1.33	5	1.1	1.23
	20 (CP)	*4*	*11.0*	*1.22*	*4*	*11.8*	*0.96*
48	0	4[1]	1.8	1.34	5	2.3	1.60
	2000	5	1.4	1.09	4[1]	2.0	1.05
	20 (CP)	*2*[2]	*18.5*	*1.22*	*2*[2]	*9.3*	*0.96*

Statistically significant modification: not mentioned;
[1]: 3 animals were excluded from the analysis, as the cell content of "blast cells" (presumably from granulocyte precursors) was spread over the slides, thereby obscuring the scoring (granules could inadvertently be scored as MN);
[2]: 2 animals were erroneously sacrificed at 48h i.o. 24h post-treatment

Conclusion: Kresoxim-Methyl was not clastogenic or aneugenic in these experimental conditions.

Guidelines: Protocol in compliance with test method B.12 of Directive 92/69/EEC
Remark: Solubility of the substance in 0.5% CMC was insufficient (as reported in a further experiment (Hanarvar, 2002)), but assumed sufficient by the notifier by mixing immediately before administration.

GLP status: The study is GLP.
Materials and methods:
In the assay, 5 rats/dose/sex [Wistar Chbb: THOM, SPF] were injected i.p. with Kresoxim-Methyl (purity 94.8%; B.n° N36) dissolved in 0.5% carboxy methyl cellulose (CMC), at the dose level of 0[1,2], 500[1], 1000[1] or 2000[1,2] mg/kg b.w. The dosing volume was 10 mL/kg. Animals were sacrificed [1]24h and/or [2]48h after dosing. An analytical verification of concentration in the administered samples was performed. Stability was checked in a sample from a comparable batch (CP5977). Homogeneity was obtained by mixing the concentrate before test sample preparation. Positive control was obtained by treating with Cyclophosphamide dissolved in a.d. (CP, 20 mg/kg, sacrifice time 24h). Negative control was obtained by treating with the vehicle. After harvest of femural bone marrow in FCS and cell smearing, slides were processed for Giemsa staining in order to distinguish polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). A minimum of 2000 cells/animal/dose were scored. The ratio of PCE to normochromatic erythrocytes (NCE) was calculated on 1000 cells as an indication of bone marrow toxicity. Incidence of micronuclei was scored among PCE's (MPCE's) and also NCE's (MNCE's). In addition a differentiation of small (<1/4 nucleus diameter) and large (>1/4 nucleus diameter) was made to recognise possible clastogenic and aneugenic events.
No *preliminary experiment* was conducted.

Criteria for determining positive response: if the mean number of MPCE was statistically different from controls in a dose-dependent way (Wilcoxon test).
The study is accepted.

B.6.4.2.2 *In vivo* unscheduled DNA synthesis test with mammalian liver cells.

-Report: *ex-vivo* unscheduled DNA synthesis(UDS) assay and S-phase response in rat hepatocytes with Reg.n°.242 009. (Pôlloth et al.,1994b, Dossier BASF)

Guidelines:
Protocol not fully in compliance with the OECD guideline (draft, 1991).
GLP status : yes
Material and methods *:*
3 male Wistar (Chbb:THOM (SPF))/dose received by gavage kresoxim-methyl (94.3%; N36) at 20; 200; 1000 mg/kg bw in aqueous suspension (CMC 0.5%). Animals were sacrificed 18h after dosing and hepatocytes were isolated. Approx. 400,000 viable cells/well were used. UDS was measured. Positive control for UDS: 2-AAF.
Deviation from the protocol: the highest tested dose is a non toxic dose. Sampling time of liver cells is too long after exposure (normally 12-16 hours after dosing).

In addition, S-phase-response, i.e. the influence of the test substance on S-phase (replicative) DNA-synthesis was measured as indicator of cell proliferation by determining the % of S-phase cells.
Positive control for S-phase response: 4-AAF.
The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate. Positive control gave the expected response.

Findings *:*
Table B.6.4.2.2-1 : *In vivo* S-phase response .

	concentration (mg/kg bw)			
	0	**20**	**200**	**1000**
Kresoxim-Methyl	1.00 ± 0.26	1.37 ± 1.07	2.78 ± 2.18	2.58 ± 1.6
4-AAF	-	-	-	5.87 ± 1.07

Expressed in mean % of S-phase cells ± SD

Kresoxim-methyl was not cytotoxic for the hepatocytes in the concentrations tested. No increase in the UDS activity was observed in either of the concentrations tested.
A dose-related increase in the main % of S-phase-cells over control values was observed after treatment with 20 and 200 mg/kg bw. No further increase is noted at a dose of 1000 mg/kg bw.(table B.6.4.2.2.1)

Conclusion *:*
Kresoxim-methyl is considered to be inactive in the UDS assay under the given test conditions but has some cell proliferating activity due to the dose-related trend shown.
NOAEL: 20 mg/kg bw

-Report: *ex-vivo* unscheduled DNA synthesis (UDS) assay in rat hepatocytes with Reg.n°.242 009 after administration in the diet for 3 weeks. (Pölloth et al.,1994c, Dossier BASF)

Guidelines:
Protocol not fully in compliance with the method of the OECD guideline (draft,1991)
GLP status : yes
Material and methods :
3 male Wistar rats (Chbb:THOM (SPF))/dose received in their diet kresoxim-methyl (94.3%; B.n° N36) at 200 or 16000 ppm (corrected dose: 10 or 800 mg/kg bw) for 3 weeks. Animals were sacrificed 16 h after the end of exposure. Approx. 400,000 viable hepatocytes/well were used . Positive (2-AAF) and negative control (gavage

with CMC(5%) were treated once by gavage 18 h prior sacrifice. The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate.
Deviation from the protocol: the highest tested dose is a non toxic dose.
Findings *:*
None of the dose groups showed repair activity nor hepatotoxicity.
Conclusion *:*
Kresoxim-methyl does not induce DNA repair in primary rat hepatocytes after 3 weeks *in vivo* exposure.

B.6.4.3 *In vivo* studies in germ cells (Annex II A 5.4.3)

Due to the negative results obtained in the *in vitro* tests as well as in the *in vivo* tests on somatic cells, no studies are warranted in germ cells. However, notifier submitted an *in-vivo* study.

-Kresoxim-methyl, oral (gavage), mouse spermatogonial cells, chromosomal aberration *in-vivo*, 500-2000 mg/kg b.w. (Honarvar, 2002)

Findings:
In the *preliminary experiment*, hypoactivity (until 4h post-dose) and ruffled fur (until 6h post-dose) was observed at 2000 mg/kg b.w., confirming this dose to be the high dose for the main experiment.
In the *main experiment*, 5/6 animals and 2/6 animals showed hypoactivity at 1h and at 2-4h post-dose, respectively.
The treatment with the test article was without effect on the number chromosomal aberrations, at any dose or at any time-point. The mitotic index was unaltered. In contrast, the positive control induced a significant number of cells showing gaps, breaks, fragments and exchanges, and reduced the M.I. with about 26% compared to the control level.

Table B.6.4.3-1: Aberration incidence in mouse spermatogonial cells treated with Kresoxim-Methyl

Sampling time (h)	Dose (mg/kg bw)	% aberrant cells		Mitotic Index (%)	
		+ gaps	– gaps	mean	relative to ctrl
24	0	0.8	0.6	5.26	100.0
	500	0.6	0.4	5.20	98.9
	1000	0.8	0.6	5.34	101.5
	2000	0.4	0.4	5.80	110.3
	50 (ADR)	7.6	6.4**	3.88	73.8
48	2000	1.0	0.8	6.54	124.3

N=5 animals/dose; Statistically significant modification: **: p≤0.01 (Mann-Whitney test)

Conclusion: Kresoxim-Methyl was not clastogenic in germ cells in these experimental conditions.

Guidelines: Protocol in compliance with test method B.23 of Directive 92/69/EEC

GLP status: The study is GLP.
Materials and methods:
In the *main assay,* 6 ♂mice/dose [NMRI] were administered a single oral dose of Kresoxim-Methyl (purity 94.8%; B.n° N36) dissolved in corn oil, at the dose level of 0[1], 500[1], 1000[1] or 2000[1,2] mg/kg b.w. by gavage The dosing volume was 10 mL/kg. Animals were sacrificed [1]24h or [2]48h after dosing. Prior (5h) to sacrifice, animals received an i.p. injection with colcemid (4.0 mg/kg b.w.) to obtain metaphase arrest. An analytical verification of concentration in the administered samples was planned (aliquots at –80°C prepared) and reported elsewhere (the substance was stable within 4h after preparation). Homogeneity was tested and insufficient in CMC and PEG, and found good in corn oil. Positive control was obtained by injecting Adriblastin (Doxorubicin.HCl) dissolved in 0.9% NaCl solution (ADR, 5 mg/kg, sacrifice time 24h) by i.p.. Negative control was obtained by treating with the vehicle. After harvest of testis tubular cells in 5 mice/dose (collagenase treated) and appropriate processing, slides were stained for Giemsa. A minimum of 100 metaphases/animal/dose were scored for chromosome analysis (on cells with 40±2 chromosomes/nucleus), and 1000 cells/animal/dose scored for M.I. determination.
A *preliminary experiment* was conducted on 2 animals treated at 2000 mg/kg b.w. to determine the toxicity.
Criteria for determining positive response: if the mean number of cells with aberrations was rised clearly or in a dose-dependent way above control levels (the latter being in the range of HCD, i.e. 2% aberrant cells excluding gaps) in at least one test point. Statistically significant results (Mann-Whitney test) confers supportive information.
The study is accepted.

B.6.4.4 Summary of genotoxicity (Annex IIA 5.4)

Table B.6.4.4-1 : Summary of genotoxicity of kresoxim-methyl

Type of test	Result	kresoxim-methyl purity, B.n°	References
***In vitro* genotoxicity tests:**			
Ames test TA1535, TA1537, TA98 and TA100+/- S9+ E.coli WP2uvrA	negative	93.7%; B.n°:N27	Gelbke et al.,1993
Ames test TA1535, TA1537, TA98 and TA100 +/- S9	negative	94.3%; B.n°:N36	Gelbke et al.,1994
HPRT locus assay in CHO-K1 cells	negative	94.3%; B.n°:N36	Pölloth and Hoffmann, 1994
chromosomes aberrations on human lymphocytes+/- S9	negative	98.7%; B.n°:N21	Engelhardt and Hoffmann,1993
UDS in mammalian cells	negative	94.3%; B.n°:N36	Pölloth and Hoffmann, 1994a
***In vivo* genotoxicity tests:**			
micronucleus assay in mice (i.p exposure)	negative	93.7%; B.n°:N36	Engelhardt and Hoffmann,1993a
micronucleus assay in rat (gavage)	negative	94.8%; B.n°:N36	Engelhardt,1997
rat hepatocytes (unique oral adm.) : UDS	negative	94.3%; B.n°:N36	Pölloth and Hoffmann, 1994b
rat hepatocytes (unique oral adm.): proliferating activity	positive at >200 mg/kg bw	94.3%; B.n°:N36	
UDS in rat hepatocytes(oral adm.:3 wks)	negative	94.3%; B.n°:N36	Pölloth at al.,1994c
chromosome aberration assay in rat spermatogonia (i.p.)	negative	95%; B.n°:N36	Honarvar, 2002

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

B.6.5.1 Long-term (2 years) oral toxicity in the rat (Annex IIA 5.5)

-Report: Chronic toxicity study with Reg.n°.242 009 in Wistar rats. Administration in the diet for 24 months. (Mellert et al.,1994c, Dossier BASF)
Supplement: Pathology report (Pappritz, 1994)
Guidelines:
Protocol not fully in compliance with the method B.30 of Directive 87/302/EEC or OECD 452 (1981).
GLP status : yes
Material and methods :
20 Wistar (Chbb: THOM) rats/sex/dose received in the diet kresoxim-methyl (B.n°.N27, 94%; B.n°.N30, 96.6%; B.n°.N36, 93.7%) at 0, 200,800, 8000 and 16000 ppm for about 24 months.
Mean daily test substance intake: 0, 9, 36,370 and 746 mg/kg/d for male
0, 12, 48, 503 and 985 mg/kg/d for female
Findings :
Deviation from the official protocol: the activity of blood ornithine decarboxylase was not measured; ovaries were not weighed.
The main treatment related effects are given in the table B.6.5.1-1. The MTD was reached.

Table B.6.5.1-1 : Long-term (2 years) oral toxicity in the rat.

endpoint/dose	**0**		**200 ppm**		**800 ppm**		**8000 ppm**		**16000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
cumulative mortality:										
day 679	10%	10%	25%	20%	25%	35%	15%	20%	10%	15%
day 707	15%	15%	40%	20%	25%	35%	15%	20%	20%	15%
day 735	15%	25%	60%	25%	25%	40%	20%	20%	20%	15%
clinical signs:			no treatment related effects							
food consumption:			no treatment related effects							
body weight							↘5%, d 42	↘10%	↘5%, d 42	↘10%
body weight gain							↘10%	↘15%	↘10%	↘15%
ophthalmology			no treatment related effects							
hematology			no treatment related effects							
clinical chemistry:										
AP			↘	↘	↘	↘	↘	↘	↘	↘
ALT									↘	
GGT							↗		↗	
urine analysis			no treatment related effects							
organ weight:										
liver							↗ r15%		↗r ,a 20%	
Statistically significant increase (↗)or decrease (↘) (p<0.05); ANOVA +Dunnett's test (two-sided); d:day; r:relative; a:absolute										
non-neoplastic microscopic findings in liver:										
-eosinophilic	0/20	1/20	1/20	0/20	0/20	0/20	6/20	0/20	8/20	1/20

endpoint/dose	0		200 ppm		800 ppm		8000 ppm		16000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
foci		(1S)	(1D)				(1D,5S)		(8S)	(1S)
-hepatocellular hypertrophy	0/20	1/20 (1S)	0/20	1/20 (1S)	3/20 (3D)	0/20	4/20 (2S,2D)	1/20 (1S)	7/20 (6S,1D)	8/20 (8S)
observed in number (S):survivors ; (D):decedent										
neoplastic microscopic findings in liver:										
-hepatocellular carcinoma	0/20	1/20 (1D)	1/20 (1D)	0/20	1/20 (1S)	2/20 (1S,1D)	3/20 (3S)	6/20 (6S)	8/20** (7S,1D)	6/20 (5S,1D)
total M+F	1/40		1/40		3/40		9/40*		14/40***	
cholangiocarci-noma			2/20 (1S1D)							
-rats with hepato. carc. or cholan-giocarcinoma	0/20	1/20	3/20	0/20	1/20	2/20	3/20	6/20	8/20**	6/20
total M+F	1/40		3/40		3/40		9/40*		14/40***	
Chi-Squared Analysis:* < 0.05 ** < 0.01*** < 0.001; observed in number (S):survivors ; (D):decedent										

Comments:
The decreases in AP, between 10 and 40% of the value in the control group, and the decreases in ALT between 15-30% were not dose-related and remain difficult to explain.
Food intake remained within normal limits and fasting can therefore not explain the decrease in AP.
- *The pre-neoplastic findings in the liver were* eosinophilic foci, often associated with slight to severe spongiosis hepatis or angiectasis. The incidence of minimal to moderate hepatocellular hypertrophy was increased in males and females of the high dose group. The non-neoplastic liver lesions-eosinophilic foci of hepatocellular alteration and hypertrophy of hepatocytes further indicate metabolic stress on the liver parenchyma.
- *Neoplastic microscopic findings in the liver* : significant increase in malignant liver tumours in groups 8000 and 16000 ppm.
The primary epithelial neoplasms of the liver were cholangiomas, carcinomas, and cholangiocarcinomas. The statistically significant increase in liver carcinoma substantiated the necropsy findings of liver masses in males and females of group 8000 ppm and 16000ppm.
Kresoxim-methyl, in high doses, is therefore a carcinogen in the rat. These neoplastic lesions, however, had no impact on animal survival. The pre-neoplastic lesions, together with an absence of genotoxic potential (see B.6.4) confirm the interpretation, already advanced in the subchronic tests, that this is due to a metabolic stress on the liver parenchyma.
Conclusion:
Kresoxim-methyl is carcinogenic at the high doses, but without impact on animal survival.
NOEL = NOAEL=800 ppm=36mg/kg bw/d for males and 48 mg/kg bw/d for females.
LOAEL for liver lesions and carcinogenicity = 8000 ppm = 370-503 mg/kg bw/d

B.6.5.2 Carcinogenicity study in the rat (Annex IIA 5.5)

- Report : carcinogenicity study with Reg.n°.242 009 in Wistar rats. Administration in the diet for 24 months. (Mellert et al., 1994d, Dossier BASF)
Supplement: Pathology report (Pappritz,1994a)
Guidelines:
Protocol not fully in compliance with the method B of the Directive 87/302/EEC or OECD 451 (1981).
GLP status : yes
Material and methods :
50 Wistar (Chbb: THOM) rats/sex/dose received in the diet kresoxim-methyl (B.n°.N27, 94 %; B.n°.N30, 96.6%; B.n°.N36, 93.7%) at 0, 200, 800, 8000 and 16000 ppm for about 24 months
Mean daily test substance intake: 0, 9, 36,375 and 770 mg/kg/d for male
0, 12, 47, 497 and 1046 mg/kg/d for female
Findings :
Deviation from the official protocol: blood smears were prepared only at the end of the study and for the highest dose group.
The findings concerning survival, clinical signs, food consumption, body weight, ophthalmology, hematology, clinical chemistry and urine analysis were essentially the same as in the previous test, occurring at the same dose levels.
Oncogenic potential : tumours and figures are given in the table B.6.5.2.1.
- *Pre-neoplastic findings* in the liver in the high dose group : foci of hepatocellular alteration was increased in females, increased incidence and degree of severity of eosinophilic foci in males of this group but decreased incidence and degree of severity of basophilic foci. The altered foci were often associated with slight to severe spongiosis hepatis or angiectasis, which were considered the histologic correlate to many of the cysts recorded at necropsy. The incidence and degree of severity of minimal to severe bile duct proliferation were increased in female, while the incidence of fatty change was decreased.
- *Neoplastic findings in the liver of the high dose groups*: cholangiomas, adenomas, carcinomas and hepatocholangiocarcinomas, females being more severely affected than males.
These tumours predominantly occurred in animals that survived until the scheduled termination of the study. The majority of liver tumours was recorded in animals that were sacrificed at scheduled termination of the study, only 3 animals appeared to die before the end of the study because of their tumour. Of all liver carcinomas, three had metastasized (lung carcinomas, mediastinal lymph node carcinomas).

Comments:
This study largely confirms the findings of the previous one indicating that kresoxim-methyl, at high doses applied during a long time, imposes metabolic stress on the liver and bile duct leading to pre-neoplastic and neoplastic lesions. The assumption of a concurrent stimulation of cell proliferation in both liver and bile duct cells at the high dose levels was also supported by the increased incidence and severity of bile duct proliferation in females of the high dose group.

Conclusion:
Kresoxim-methyl is carcinogenic at the high doses, but without impact on animal survival.
NOEL = NOAEL= 800 ppm=36 mg/kg bw/d for males and 47 mg/kg bw/d for females.
LOAEL for liver lesions and carcinogenicity = 8000 ppm = 375-497 mg/kg bw/d

Table B.6.5.2-1 : Carcinogenicity study in the rat.

non-neoplastic microscopic findings in liver:										
endpoint/dose	**0**		**200 ppm**		**800 ppm**		**8000 ppm**		**16000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
-eosiniphilic foci of hepatocel. alter.	1/50	3/50	0/50	0/50	3/50	3/50	5/50	8/50	11/50 (stat.sig.)	5/50 (stat.s.)
-bile duct prolife-ration		10/50		13/50		12/50		13/50		28/50 (stat.s.)
neoplastic microscopic findings in liver: **(Chi-Squared Analysis:* <0.05 ***<0.01)**										
surviving animals	32	31	36	35	35	39	36	36	35	38
hepatocellular adenoma	1	0	0	1	0	2	1	2	0	1
hepatocellular car-cinoma	7(2S, 5D)	1(S)	5(4S, 1D)	1(1D)	2(2S)	2(2S)	18* (16 S, 2D)	13*** (10 S, 3D)	11 (8S,3D)	16*** (16S)
combined inci-dence be-nign+malignant hepatocel.neo	8	1	5	2	2	4	19*	15***	13	17***
cholangiomas	0	0	0	0	1	0	0	1	1	2

Statistically significant increase (↗)or decrease (↘) ($p<0.05$); F test (ANOVA)+Dunnett's test.
S: surviving animals; D : decedent animals.

At the occasion of the re-submission, the notifier presented the independent evaluation of the liver neoplastic findings (histopathology) by the Pathology Working Group (PWG)
The findings of the chronic toxicity (n=20) and the carcinogenicity study (n=50), were presented in table B.6.5.2-2, B.6.5.2-3, and B.6.5.2-4
(Hildebrandt, 1995a and 1995c)

Table B.6.5.2-2 : Carcinogenicity study in the rat (re-evaluation of neoplastic findings in all previous studies by the PWG)

endpoint/dose	0		200 ppm		800 ppm		8000 ppm		16000 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Chronic toxicity study (n=20)										
hepatocellular adenoma	0	0	0	0	0	1	1	4	4	4
hepatocellular carcinoma	0	0	1	0	1	1	3	2	3	3
combined incidence (ad+carc)	*0*	*0*	*1*	*0*	*1*	*2*	*4*	*5**	*7***	*6***
Carcinogenicity study (n=50)										
hepatocellular adenoma	2	0	2	2	0	4	5	11**	4	11**
hepatocellular carcinoma	4	1	4	0	2	1	12*	7**	8	7**
cholangiocellular carcinoma	0	0	0	0	0	0	0	0	1	0
combined incidence (ad+carc)	*6*	*1*	*6*	*2*	*2*	*5*	*16*	*16***	*11*	*17***
Combined incidence chronic toxicity + carcinogenicity study (n=70)										
hepatocellular adenoma	2	0	2	2	0	5*	6	15**	8*	15**
hepatocellular carcinoma	4	1	5	0	3	2	15**	9**	12*	10**
combined incidence (ad+carc)	*6*	*1*	*7*	*2*	*3*	*7**	*20***	*21***	*18***	*23***

Statistically significant modification (*p≤0.05; **p≤0.01); one-sided Fisher's exact test.

The adenoma incidence at 800 ppm in the combined evaluation of the long-term rat studies was 5 out of 70♀(about 7%). The finding attained statistical significance (p≤0.05, without reaching the statistical 1% level, which was considered by the PWG to be required for this common neoplasia, because the control incidence was unusually low (0%). Contemporary historical control data for liver adenomas in ♀Wistar rats in BASF's laboratories (N= 38 studies) was 43/1620 animals (2.7%). This indicates that normally in a group of 70 female control animals, approx. 3 would have been found to have a liver adenoma. According to the notifier, there is no statistical, nor biological, significance between 3/70 (the historical mean) and 5/70 animals (the actual number at 800 ppm). Moreover, the historical data of BASF's laboratories also demonstrate that the maximum incidence of liver adenoma in control ♀Wistar rats can be as high as 20%, indicating that the 7% noted in the Kresoxim-methyl studies at 800 ppm was well within the historical range. Similarly, the incidence of combined liver adenomas and carcinomas in the controls (1.4%) was also lower than the historical mean of 3.4%. Again the actual study incidence at 800 ppm (10%) is well within the historical range (up to 22%). Therefore, it is concluded that an oncogenic potential on the liver of Wistar rats only exists at dose levels of ≥8000 ppm. The RMS agrees with this position.

New study added at re-submission (05/2009):

-Rat, Kresoxim-Methyl in diet, 0 or 16000ppm for 24 months (Kamp, 2008)

Report: BAS 490 F (Kresoxim-Methyl) Carcinogenicity study in Wistar rats – Crl GlxBrI Han: WI) Administration via the diet over 24 months (Kamp et al., 2008)

Findings:
Mortality: was not increased in the treated group.
Clinical signs: No treatment-related changes.

Food consumption, bw and bw gain:
The food consumption was significantly decreased in all animals (♂: up to 8% during wk 0-9; ♀: up to 14% throughout the experiment).
The body weights were marginally low in the ♂ (up to 6% lower) consistently until wk25 and sporadically thereafter. The ♀ were up to 16% lighter than the controls throughout the study. At termination, the body weight change was decreased both in the ♂ (-6%) and in the ♀ (-24%).
Overall, both food consumption and body weight effects were thus more pronounced in the ♀ than in the ♂.

Table B.6.5.1-2a: carcinogenicity study of Kresoxim-Methyl in rats: body weight effects (Kamp, 2008):

Dose (ppm)		**0**		**16000**	
		♂	♀	♂	♀
Food consumption	d0-63			↓3%*-8%**	
	d728			↓11%**	
	d0-d707				↓4*-14%**
Body weight	d21-175			↓4*-6%*	
	d14-728				↓5%**-16%**
Body weight gain	d0-175			↓8%**-12%**	
	d175-728			↓3%-8%*	
	d0-728				↓11%*-27%**

Statistically significant modification, *p≤0.05 or **p≤0.01(Welch t-test).

Clinical chemistry: not performed.
Organ weight:
Both significant (p≤0.01) increases of absolute (+15%) and relative (+20%) liver weights in the ♂ were considered treatment-related, as terminal body weight was only decreased by 4% (n.s.s.). In addition, the finding was in line with observed hepatic lesions. The increased relative weight of adrenals, brain, heart, kidneys, ovaries and uterus in the ♀ was probably associated with the concomitant decreased terminal body weight. The relevance of the significant relative liver weight increase in the ♀ was unclear, in the absence of a concomitant absolute weight increase.

Gross pathology:
The number of macroscopically discolored foci and masses was increased in both the ♂ and in the ♀. Other gross lesions were equally distributed among control and treated animals.

Table B.6.5.1-2b: carcinogenicity study of Kresoxim-Methyl in rats: liver weights and necropsy (Kamp, 2008):

Dose (ppm)		**0**		**16000**	
		♂	♀	♂	♀
Liver weight	a r			↑15%** ↑20%**	- ↑16%
Gross liver lesions (/ 50)	discoloured foci	17	13	40	32
	masses	3	2	10	5

a: absolute; r: relative; Statistically significant modification, *p≤0.05 or **p≤0.01(Wilcoxon test).

Histopathology:

Non-neoplastic findings:

Treated animals showed increased incidences of foci of cellular alterations. Incidences of eosinophilic foci and of non-diffuse, non-tigroid basophilic foci were observed. In addition, treated animals exhibited significant focal alterations with spongiosis hepatis or peliosis. Other lesions such as single cell fatty change or peripheral hepatocellular hypertrophy were also considered treatment-related. The single-cell degeneration was only s.s. in the ♀, but the notfier considered the event incidental. Other fatty changes of (multi)focal, perifieral or diffuse character were unremarkable.

Neoplastic findings:

There was an increased incidence of hepatocellular adenoma in both the ♂ and the ♀ when compared to the study control. The incidence of hepatocellular carcinoma was clearly increased in the ♂, and slightly increased in the ♀. For the ♀, notifier highlighted that the study incidence of carcinoma was within the HCD (6%). However, in the HCD, there was only 1 out of 7 studies where 3/50 spontaneous carcinoma were observed (in exp. 99024, conducted 08.99-08.01, thus not in the 5 year window for HCD). Other incidences were maximally 1/50 (n=2) and otherwise 0/50 (n=4). In the light of the presence of other preneoplastic lesions, and knowing that overall, the liver was the target organ, the incidence of malignant tumours in both sexes should be regarded treatment-related.

Table B.6.5.1-2d: carcinogenicity study of Kresoxim-Methyl in rats-histopathology (Kamp, 2008):

Dose (ppm)		**0**		**16000**	
		♂	♀	♂	♀
Non-neoplastic findings					
Focus of cellular alteration		40	38	44	47*
Clear-cell		14	4	18	0
Eosinophilic		26	7	41**	30**
Basophilic	tigroid	28	35	19	36
	diffuse	11	4	9	2
	NOS	2	0	6	14**
Foci with spongiosis/peliosis		1	2	10**	16**
Fatty change, single cell		21	19	23	29*
Periportal hypertrophy		0	0	6*	12**
Neoplastic findings					
Hepatocellular adenoma		0	0	3	4
Hepatocellular carcinoma		3	1	13**	3

N=50/sex/dose; NOS: not otherwise specified;
Statistically significant modification, *p≤0.05 or **p≤0.01(Fisher's exact test).

Wistar rat historical control incidences of liver tumours *(remark: the HCD do not match the time-window of the study)*
[data breeder (Charles River DE) of test facility, 7 studies 1999-2007, N= 350]:

Hepatocellular adenoma: ♂, range 0-4% (mean 2.0%); ♀, range 0-6% (mean 0.9%)
Hepatocellular carcinoma: ♂, range 0-4% (mean 0.9%); ♀, range 0-6% (mean 1.4%)
Combined (Ad. + Carc): ♂, range 0-6% (mean 2.9%); ♀, range 0-6% (mean 2.3%)

In the following table, the liver tumour incidences of the former study (Mellert, 1994) were provided, as a comparison

Dose (ppm)	**0**		**16000**	
	♂	♀		♂
Neoplastic findings				
Hepatocellular adenoma	1	0	0	1
Hepatocellular carcinoma	7	1	11	16***

N=50/sex/dose; Statistically significant modification, *p≤0.05 or **p≤0.01(Fisher's exact test).

Conclusion:
Toxicity NOAEL < 16000 ppm
LOAEL= 16000 ppm = 752.1 mg/kg bw/d
based on ↓body weight (gain), ↑liver weight, ↑liver PP hypertrophy and focal alterations
carcinogenicity NOAEL < 16000 ppm
LOAEL= 16000 ppm = 752.1 mg/kg bw/d
based on ↑liver carcinoma

In the former 2 year feeding study, conducted on another strain of Wistar rats, it was concluded that the occurrence of liver tumours was related to the treatment with Kresoxim-methyl. The study was replicated in another strain of Wistar-rats at the control and top-dose, under the assumption that the latter strain would be less sensitive to the induction of liver tumours.
The data do not support this assumption, although the liver carcinoma incidence in the new study was significantly lower in the ♀, as compared to the former study. However, when the incidences of both the ♂ and the ♀ are considered, it would appear that the difference between the two studies was not impressive (old study: 27 in the treated vs. 8 in the controls; new study: 16 in the treated vs. 4 in the controls).

Therefore, the new results did not alter the former conclusion, being that Kresoxim-methyl was to be considered carcinogenic towards the rat (Carc. Cat. 3; Xn; R40).

Guidelines: Protocol partly in compliance with test methods B.33 of directive 88/302/EEC
Deviation from protocol:
only control and top-dose level examined;no intermediate blood sampling or urinalysis; no interim kill; no clinical chemistry; acceptable as complementary experiment of previous full rat carcinogenicity study.

Justification of this complementary study:
In earlier long-term rat studies, performed in another Wistar rat strain (Chbb:Thom), an increased liver tumour incidence was observed at the dose-levels of 8000 and 16000 ppm. This strain was considered by the notifier to have an unusually higher sensitivity for the development of spontaneous preneoplastic foci and of liver neoplasia, as compared to the Wistar rats from another origin. Notifier further considered that another Wistar rat strain (Crl Glx Brl Han:WI) was more suitable for the extrapolation of the results to human, based on its profile of development of spontaneous liver tumours.
The objective of the present study was to determine whether the test substance exhibited a carcinogenic potential when administered to this latter strain.

GLP status: The study is GLP

Materials and methods:
Fifty rats/sex/dose (Crl Glx Brl Han:WI)) were fed a diet of Kresoxim-Methyl (purity 97.8%; B.n° COD-000225) at the dose levels of 0 or 16000 ppm. At termination, blood was collected for haematologic analysis (no clinical chemistry) from all rats.
Analytical verification revealed dose levels being in expected ranges of nominal values during the whole treatment period.
Compound stability and homogeneity in the diet proved to be satisfactory . Achieved test article intake: 752.1(♂)-1021.6(♀) mg/kg bw/d. The study is accepted.

B.6.5.3 Carcinogenicity study in the mouse (Annex IIA 5.5)

-Report: carcinogenicity study with Reg.n°.242 009 in C57BL mice. Administration in the diet for 18 months. (Mellert et al.,1994e, Dossier BASF)
Guidelines:
Protocol in compliance with the method B of the Directive 87/302/EEC .
GLP status : yes

Material and methods :
Satellite groups of 10 C57BL/6N CrIBR mice/sex/dose received in the diet kresoxim-methyl (B.n°.N30, 95.4%; B.n°.N36, 92.7%) at 0, 400, 2000 and 8000 ppm for about 12 months .
Main groups of 50 C57BL/6N CrIBR mice/sex/dose received in the diet kresoxim-methyl (B.n°.N30, 95.4%; B.n°.N36, 92.7%) at 0, 400, 2000 and 8000 ppm for about 18 months.

Mean daily test substance intake: 0, 60,304 and 1308 mg/kg/d for males.
0, 81, 400 and 1662 mg/kg/d for females.

Findings :

Satellite group: interim kill at 52 weeks.

Body weight and bw gain were reduced in the high dose group (approximately 11% and 20% respectively). There was a reduced brain weight in males, unrelated to dose and not seen in the main study, therefore considered as not substance related. The weight of the testes was lowered in the low dose group, that of the adrenals in the high dose males but without histopathological correlation. Liver weight was increased in the high dose females. There were no further treatment related effects observed. The number of tumours, which was very low, was not different between non-treated and treated animals.

Main study: sacrifice after 78 weeks.

The main treatment related effects are given in the table B.6.5.3.1. The MTD was reached.

Table B.6.5.3-1 : Carcinogenicity study in the mouse.

endpoint/dose	**0**		**400 ppm**		**2000 ppm**		**8000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mortality in %	14	20	12	12	16	18	18	16
clinical signs								
food consumption								
body weight						↘11%	↘9%	↘20%
body weight gain						↘22%	↘21%	↘39%
ophthalmology								
hematology:								
clinical chemistry								
urine analysis								
organ weight:								
kidneys							↗r	
testes							↗r	
adrenal glands						↘a	↗r	↗ r
brain								↘a ,↗r
liver								↗r
kidneys retraction		4/50				2/50		13/50
kidneys: papillary necroses	1	2	2	4	3	4	1	13*
liver: amyloidosis	13	6	11	1	7	4	20	16*
foci of cellular alterations	3	1	3	1	3	1	7	2
hepatocellular adenomas			1		1		1	3
hepatocellular carcinoma	4	1	3	1			3	

Statistically significant increase (↗) or decrease (↘) (*p≤0.05); F test (Anova) + Dunnett's test. r: relative; a :absolute

Comments:

The changes in weight of kidneys, testes, brain and adrenals are the result of the reduced terminal body weight. Moreover, no abnormal histopathological findings were seen in these organs. Retraction in the kidneys in most cases was accompanied by amyloidosis and occurred mostly in surviving females. The number of animals with amyloidosis of the kidneys was comparable between control and treated groups. A higher incidence of papillary necroses in the top dose females indicates that the kidney can also be a target organ.

Amyloidosis, a well known geriatric alteration in C57BL-strain, occurred in liver of males and females : in males the degree of severity was comparable between control and treated animals, but in females, the animals of the high dose group showed higher degree of amyloidosis. The increased relative liver weight in the top dose females of the main and satellite groups was not associated with histopathological signs .
The number of animals with neoplasms, the number of animals with benign, malignant and systemic neoplasms as well as the total number of benign, malignant and systemic neoplasms were comparable between control and treated animals.
Conclusions :
The target organs at the high dose, therefore, are the liver and the kidney. In the mouse kresoxim-methyl, at the doses studied, was not oncogenic.
NOEL = NOAEL = 400 ppm = 81 mg/kg bw/d for females and 304 mg/kg bw/d (2000 ppm) for males.
LOAEL = 2000ppm = 400 mg/kg bw/d for females and 1308 mg/kg bw/d (8000 ppm) for males.

B.6.5.4 Oral long-term toxicity in the dog (Annex IIA 5.5)

No data, not necessary.

B.6.5.5. Mechanism of action and supporting data

B.6.5.5.1 Cell proliferation studies in rats.

- S-Phase-response study with Reg.n°.242 009 in Wistar rats after administration in the diet for 3 weeks. (Pölloth et al.,1994d, Dossier BASF)

Material, methods and findings:
5 male Wistar rats (Chbb: Thom (SPF))/dose received in their diet kresoxim-methyl (94.3%, B.n°.:N36) at 0, 200 and 16000 ppm for 3 weeks (mean daily intake: 15, 1140 mg/kg bw). One week prior to necropsy, osmotic minipumps, filled with bromodeoxyuridine, were implanted subcutaneously. Bromodeoxyuridine incorporated in the DNA of the S-phase-cells was detected by immunohistochemistry and the hepatic zonal distribution evaluated microscopically.
No substance-related effects were observed for body weight, food consumption and clinical parameters.
The pathological examinations revealed the following treatment related alterations:
At 16000 ppm, statistically significant increase of cell proliferation in the hepatic lobule (Rappaport zone 1, 2 and partly 3) with the most distinct increase in zone 1. No effect on absolute or relative liver weight and no treatment related gross lesions or microscopic findings.
At 200 ppm or 15 mg/kg bw, no effects were observed.
The study is GLP.

- S-Phase-response study with Reg.n°.242 009 in 16-month old Wistar rats after administration in the diet for 3 weeks.(Pölloth et al.,1994e, Dossier BASF)

Material, methods and findings:
5 male 16-month old Wistar rats (Chbb: Thom (SPF)) /dose received in their diet kresoxim-methyl (94.3%, B.n°.:N36) at 0, 200 and 16000 ppm for 3 weeks. One week prior to necropsy, osmotic minipumps, filled with bromodeoxyuridine, were implanted subcutaneously. Bromodeoxyuridine incorporated in the DNA of the S-phase-cells was detected by immunohistochemistry and the hepatic zonal distribution evaluated microscopically.

Enhanced liver cell proliferation was observed at a dose of 16000 ppm . The zonal distribution of increased cell proliferation may reflect a greater susceptibility of hepatocytes in zone 1, possibly due to the functional blood flow in the liver, which, coming from the gastrointestinal tract, reaches zone 1 first.
No substance-related effects were observed for body weight, liver weight, macroscopy and histopathology.
At 200 ppm, no effects were observed.
The study is GLP

-Rat, S-phase response study, liver, Kresoxim-Methyl, 800-8000 ppm, 3 weeks in diet (Mellert, 1997)

- **S-phase-response study with BAS 490 02 F (Reg.No. 242 009) in Wistar rats after administration in the diet for 3 weeks (supplementary study to project No. 83M0180/910149); BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep.; 1997/10318 (Mellert, 1997)**

Findings:

Mortality: none
Food consumption: significantly ($p \leq 0.05$) decreased (-6%) on d7 in the top-dose animals.
Body weight: no relevant findings.
Clinical signs: no relevant findings.
Organ (liver) weight: no relevant findings.
Necropsy and histopathology: no relevant findings.

Determination of cell proliferation:
Enhanced liver cell proliferation was observed at a dose of 8000 ppm . At the top-dose, the labelling index of hepatocytes from zone 1 (periportal) was 69-93% higher than that of control cells, with an average of 80% increase. When periportal and intermediate cells were screened together for the LI, the increase of the top-dose animals was 19-46% above controls (36% average increase). No data about zone 3 were reported.
Thus, the proliferation was mainly confined to the periportal hepatocytes.

At 800 ppm, no effects were observed.

Conclusion:
The NOAEL was 800 ppm = 61 mg/kg b.w./d, based upon the increased cell proliferation at 8000 ppm = 603 mg/kg b.w./d.

Material and methods
Five ♂Wistar rats/dose (Chbb: Thom (SPF), about 9 weeks old) received Kresoxim-methyl (purity 94.9%, B.n°.: N36) in their diet at 0, 800 or 8000 ppm for 3 weeks, corresponding to a test article intake of 0, 61 or 603 mg/kg b.w./d. One week prior to necropsy, osmotic minipumps, filled with bromodeoxyuridine, were implanted subcutaneously. Bromodeoxyuridine, incorporated in the DNA of the S-phase-cells, was detected immunohistochemically (α-BUdR) in the liver and the jejunum (positive control). Tissue from 3 liver lobes (lobus dexter lateralis and medialis, processus caudatus) was sampled. The hepatic zonal distribution (central, periportal and intermediate) in the liver was evaluated microscopically. The labelling index (LI) was determined on ≥2000 cells/lobular zone (i.e. ≥6000 cells/animal).
The study is GLP and acceptable.

- Reg.n°.242009 -S-phase response study in male Wistar rats including reversibility. Administration in the diet up to 13 weeks (Mellert et al.,1996, Dossier BASF)

Material methods and findings:
kresoxim-methyl (N36; 92.7%)was administered to groups of 5 male Wistar rats (Chbb: Thom (SPF)) in a dietary concentration of 16000 ppm for 1, 6 or 13 weeks. At two times (1 and 13 weeks) recovery of two and five weeks, respectively were included. A slight impairment of food consumption and body weight was observed in the treated animals. Enhanced liver cell proliferation was observed in male rats after treatment for 1, 6 or 13 weeks. Compared to the controls (100%), the cell proliferation increase peaked after one week (171%). This effect was congruent with the increase of the absolute and relative liver weights. After week 6, the cell

proliferation level was at 149% and after 13 weeks at 137%. This means that the test compound enhances DNA synthesis for a period of at least 3 months. The periportal area (zone 1) showed a pronounced increase in the labeling index, especially after 6 and 13 weeks of administration. After 1 week cell replication was increased in all three zones (the smallest morphological liver unit is subdivided into 3 zones in accordance with the system of Rappaport) to a similar extent. When treatment continued for 6 and 13 weeks, the effect diminished in zones 2 and 3. These data indicate a time-dependent selective effect of the test substance on the hepatocytes of zone 1.
The enhancement of cell replication, caused by the test compound administered is reversible. Compared to controls, the values decreased to 43% after 1 week and to 30% after 13 weeks of administration and 5 weeks of recovery. It is important to note that they remained below the control values in the sense of a counter regulation.

The reversibility of the mechanism responsible for tumour formation is important and indicates that in the extremely unlikely event of an exposure at dose levels which resulted in a tumourigenic effect, a single or even short term exposure will not be associated with a carcinogenic risk.
The study is GLP

B.6.5.5.2 Liver foci assay studies in rats.

- Report: study of foci initiating activity of Reg.n°.242 009 in Wistar rats (Gamer et al.,1995, Dossier BASF)

Material, methods and findings:
8 groups of 10 Wistar rats (Chbb: Thom (SPF))/sex underwent partial hepatectomy and received 14 h afterwards, a single administration by gavage 2388 mg/kg bw of kresoxim-methyl for initiation. N-nitrosomorpholine (28 mg/kg bw) was used as positive control and carboxymethyl cellulose was administered as vehicle control.
Kresoxim-methyl (94.3%, B.n°.:N36 and a purified batch BAS 490F, B.n°.26833/147) were used. A 14 d recovery period followed. The test groups scheduled for promotion were fed from day 15 after partial hepatectomy for about 8 weeks with a 500 ppm mixture of phenobarbital in basal diet (promotion period). At the end of the study the animals were subjected to gross-pathological assessment, giving special attention to the liver. Histopathological evaluation of the liver was performed on H-E stained slides as well as on slides stained for Glutathione-S-transferase P (GST-P). GST-P positive foci were evaluated quantitatively (foci/cm^2 of liver tissue) in the groups receiving the test substance and in the vehicle control.
Initiating activity is indicated by the formation of foci after application of a test compound. To increase sensitivity of the assay partial hepatectomy is performed before administration of the test compound, and a standard tumour promotor (phenobarbital) is fed subsequently. Promoting activity is indicated by foci growth and by enhanced expression of foci phenotypes. Formation of foci is induced by application of a standard initiator (N-nitrosomorpholine) prior to test compound administration.
Foci of cellular alterations are considered as preneoplastic lesions in the liver. Glutathione-S-transferase placental form, which is hardly detectable in normal rat liver, is described to possess the greatest efficiency for scoring the largest number of altered foci .

Table B.6.5.5.2-1: Liver foci assay studies in rats

endpoint	unpromoted						promoted					
	control		kresoxim-methyl		NNM		control		kresoxim-methyl		NNM	
	M	F	M	F	M	F	M	F	M	F	M	F
animals with foci of cellular alteration	3/10	2/10	1/9	2/10	8/10	10/10	3/10	1/9	4/10	4/10	10/10	10/10
animals with GST-P^+ foci	8/10	6/10	4/9	4/10	10/10	10/10	8/10	5/9	7/10	5/10	10/10	10/10
max.incidence of GST-P^+ foci/examined liver	3	3	3	2	100	50	10	1	3	1	>100	50
mean number of foci/cm^2 of liver tissue*	0.23	0.17	0.12	0.12	n.m.	n.m.	0.46	0.11	0.25	0.11	n.m.	n.m.

n.m.: not measured ; *: animals without foci included as 0/cm^2

In all promoted groups except the vehicle control there was a tendency for increased absolute liver weights. The relative liver weights of the promoted N-nitroso-morpholine males and the promoted kresoxim-methyl females were statistically significantly increased. This is interpreted as promotor effect of the treatment.
The administration of kresoxim-methyl had no influence on the incidence of Glutathione-S-transferase P positive foci or foci of cellular alteration (H&E stained slides) compared to the vehicle control. The positive control (N-nitroso-morpholine) lead to a marked increase in both lesions.
Therefore it is concluded that kresoxim-methyl does not possess tumour initiating properties.
GLP status: yes.

- Report: BAS 490 F (Reg.No. 242 009): Medium-term promotion hepatocarcinogenesis study in rats
The Institute of Environmental Toxicology; Kodaira-shi Tokyo 187; Japan; 1997/10918 (Harada, 1997).

-Rat, two-stage hepatocarcinogenicity promotor study, Kresoxim-Methyl, 200-16000 ppm, 6 weeks in diet (Harada, 1997)

Findings (see table B.6.5.5.2-2):

Mortality and clinical signs:
No relevant findings. Notifier reported 0, 2, 3, 2, 2, and 2 deaths at 0, 200, 800, 8000, 16000 ppm KM and PB fed-rats due to technical errors during partial hepatectomy.
Feed consumption and body weight:
The treatment with KM was without statistical effect on food consumption and body weight, although b.w. was consistently 3-4% lower at the top-dose. In the PB-treated animals, b.w. was significantly ($p \leq 0.05$) increased 3-4% during the study (wk 4, 5, 8), and also food consumption was high (wk 3, 5, 7, 8).
Liver weight and gross pathology:
Both liver weight and visible liver enlargment was observed at 800 ppm and above. Also the animals treated with PB exhibited heavier and enlarged livers when compared to the controls.
Histopathology: not investigated, no data.
Quantitative analysis of GST-P^+ foci:
Meaningful increases of both number and area of GST-P^+-foci were recorded in livers of animals treated at 8000 ppm and higher. At this critical dose-levels, the N° was comparable to that of the animals treated with PB.

Conclusion:
Kresoxim-Methyl did act as a promotor in rat liver, which was previously exposed to a well-known initiator.

Based upon the described histochemical endpoints, its profile was comparable to that of Phenobarbital.
According to the notifier, effects of tumour-promoting activity was only observed at 8000 ppm and higher, thus disregarding the subtle effects on liver weight and visible enlargement (however, histopathological findings in order to corroborate these effects were lacking).
In the absence of histopathological examination (H&E stained slides not further examined), RMS is of the opinion that the liver effects at 800 ppm should not be ignored. The increase was at the limit of relevance, as the magnitude was just <10%, but the finding was highly statistically significant ($p \leq 0.01$), and definitely part of a dose-dependent, and thus substance-related trend.

NOAEL = 200 ppm = 10.72 mg/kg b.w./d
LOAEL = 800 ppm = 42.47 mg/kg b.w. /d, based upon ↑liver weight, and ↑liver enlargement

Table B.6.5.5.2-2: Promotor study of Kresoxim-Methyl in rats:

	Kresoxim-Methyl					PB
Dose (ppm)	**0**	**200**	**800**	**8000**	**16000**	**500**
n° animals examined	16	14	13	14	14	14
terminal b.w.		-	-	-	(↓3%)	↑3%*
Liver weight, a			**↑8.4%****	**↑26%****	**↑29%****	**↑43%****
Liver weight, r (b.w.)		-	**↑9.4%****	**↑29%****	**↑33%****	**↑39%****
necropsy:liver enlargment	0/16	0/14	**2/13**	**14/14****	**14/14****	**14/14****
GST-P foci[§] n°/cm²	12.0±2.8	15.1±3.5	14.5±2.8	18.5±4.5**	22.2±3.8**	18.0±2.3
mm²/cm²	1.16±0.32	1.48±0.34	1.39±0.43	1.71±0.47**	1.99±0.34**	2.48±0.44**

[§]: mean ± s.d.; Statistical significant modifications: Dunnett's and/or Student t-test (histochemistry): *p<0.05; **p<0.01.

Guidelines: No EC protocol is available
GLP status: The study is not GLP
Materials and methods:
The study design was based upon a two-stage initiator/promotor protocol (Ito-test).
16 ♂rats (F344/DuCrj Fischer, 8 wks old)/group received an initial single i.p. injection (? mL) with the initiator diethylnitrosamine (DENA at 200 mg/kg bw), followed by a 2 wk period of basal diet. Subsequently, animals received a 6 wk feeding period with either Kresoxim-Methyl, Sodium phenobarbital (PB, 0.05% w:v, positive control), or basal diet (negative control, only when initiated with DEN). At wk 3 after i.p. injection, a ⅔ partial hepatectomy (PH) was performed on the animals of all groups, in order to induce mitosis. Thus, there were 6 dose groups, as outlined in table B.6.5.5.2-3.
Kresoxim-Methyl (95.4%; B.n.° N112) was administered at 0, 200, 800, 8000 or 16000 ppm, which corresponded to a test article intake of 0, 10.72, 42.47, 430.6 or 886 mg/kg bw/d. PB was used as a positive control (500 ppm, equivalent to 27.99 mg/kg b.w./d). Analytical verification of diet, compound stability and homogeineity was performed. The test diets (prepared once and sored at RT) was replaced twice a week.
After sacrifice, liver was excised, weighed and processed for routine histology (H.E.-staining) or immunohistochemistry. For the latter, slides were stained with Glutathione-S-transferase placental form (GST-P) polyclonal antibodies. The number per unit area (n°/cm²) and relative area (mm²/cm²) of GST^+-foci (>200 µm diameter or about 0.0314 mm²) was determined by image analysis.

Table B.6.5.5.2-3: Initiation-Promotion hepatocarcinogenesis protocol in rats

Group	Initiation (followed by 2 wk basal diet)	Promotion (wk 2-8, including PH on wk 3)
1	DENA	basal diet
2	DENA	diet + 200 ppm Kresoxim-Methyl
3	DENA	diet + 800 ppm Kresoxim-Methyl
4	DENA	diet + 8000 ppm Kresoxim-Methyl
5	DENA	diet + 16000 ppm Kresoxim-Methyl
6	DENA	diet + 500 ppm PB

The study is accepted

6.5.5.3 Liver enzyme examination

Report: Reg.No. 242 009 - Examination of enzyme activities in the liver of Wistar rats - Administration in the diet for 3 weeks; BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep.;1996/10100 (Mellert, 1996a)

-Rat, liver enzyme analysis, Kresoxim-Methyl, 200 and 16000 ppm, 3 weeks in diet (Mellert, 1996a)

Findings (see table B.6.5.5.3-1):

Mortality and clinical signs: no relevant findings.
Feed and water consumption: no relevant findings.
Body weight (change):
The treatment with KM was without effect on the body weight, but at the top-dose, body weight gain was significantly ($p \leq 0.05$) impaired by 26% (d14) and 17% (d21) in the ♂.

Liver weight, gross pathology, histopathology : not investigated.
Clinical Chemistry:
The following modification of enzymatic activities or contents were observed in the animals of the top-dose.
-About 4-fold γ-GT activity in the ♂ animals, marginal increase in the ♀.
-Slightly increased microsomal CYP450 content in the ♂ and marginal increase in the ♀.
-Increased activity of the CYP2B-dependent PROD in both the ♂ and the ♀, marginal increase of CYP1A-dependent EROD in the ♀.

In contrast, CYP4A-dependent CN-insensitive palmitoyl-CoA oxidation and the omega-oxidation of lauric acid were unaffected or only marginally affected by the treatment, indicating that the substance has no noticeable activity as a peroxisome proliferator. The glutathione concentration in the liver also remained unaffected. This may be an indication that the substance does not markedly produce oxidative stress.

Conclusion:
The dietary administration of Kresoxim-Methyl at 16000 ppm during 3 weeks induced the CYP 2B-family of CYP450, predominantly in the ♂. The increase of γ-GT was possibly associated with liver disease (hepatocellular or cholestatic). As histopathology was not performed, the etiology is unknown. Otherwise, it could be argued that the finding was an indication of a weak PB-like induction pattern. It was of note that in former subacute or subchronic toxicity studies at this dose, overt hepatotoxicity was not demonstrated.

NOAEL = 200 ppm = 13 mg/kg b.w./d
LOAEL = 16000 ppm = 973 mg/kg b.w. /d based upon modifications in hepatic clinical chemistry parameters

Table B.6.5.5.2-2: Analysis of hepatic clinical chemistry after treatment (21d) with Kresoxim-Methyl in rats:

Dose (ppm)		**0**		**200**		**16000**	
		♂	♀	♂	♀	♂	♀
B.w. gain (d21)						↓17%*	-
γ-GT	(nkat/g prot)	44.94	97.89	43.91	81.67	216.58**	124.40
	% ↑↓					***↑382%***	*↑27%*
Pal CoA	(mU/mg prot)	2.53	2.29	2.07	2.24	2.25	1.79
	% ↑↓						
GSH	(µmol/g)	9.40	7.06	8.18**	7.04	9.02	7.75
	% ↑↓						
ω-LA	(nmol/min/mg prot)	0.345	0.425	0.369	0.462	0.536	0.550
	% ↑↓					*↑55%*	*↑29%*
CYP450	(nmol/mg prot)	0.499	0.453	0.463	0.486	0.615 ‡	0.567
	% ↑↓					***↑32%***	***↑25%***
PROD	(µmol/min/mg prot)	0.14	0.81	0.10	0.69	0.28 ‡	2.3
	% ↑↓					***↑100%***	***↑184%***
EROD	(µmol/min/mg prot)	0.72	2.39	0.45	2.27	0.68	3.82
	% ↑↓						*↑60%*

Values expressed as means (n=10).; Statistical significant modifications: Dunnett's *p<0.05; **p<0.01,or Wilcoxon test: ‡p>0.05; ‡‡p<0.01.

Guidelines: No EC protocol is available
GLP status: The study is GLP

Materials and methods:
10 rats (Wistar Chbb: THOM (SPF), 12-13 wks old)/sex/dose received Kresoxim-Methyl (94.3%; B.n.° N36) in the diet at 0, 200 or 16000 ppm, which corresponded to a test article intake of 0, 13 or 973 (♂) or 0, 15 or 1186 (♂) mg/kg b.w./d.
Analytical verification of diet was performed. Compound stability (32d) and homogeineity was not performed in this study, as this has been tested previously.
After sacrifice, liver was excised, weighed and processed for clinical chemistry. Following parameters were determined: (i) gamma-glutamyltransferase (γ-GT), cyanide-insensitive palmitoyl-CoA-oxidation (PalCoA), Cytochrome P450 (CYP450) content, omega-oxidation of lauric acid (ωLA), pentoxyresorufin-depentylase (PROD) and ethoxyresorufin-deethylase (EROD). Glutathione (GSH) concentration, and protein-content were determined in addition.
The study is accepted.

B.6.5.5.3 Conclusions concerning the mechanistical studies

Table 6.5.5.3-1 : Summary of the studies involved in the identification of the tumourigenic mechanism of kresoxim-methyl.

Endpoints	Results	Critical dose	References
S-phase response, 1 dose (20, 200, 1000 mg/kg bw)	↑cell proliferation	200 mg/kg bw	Pölloth et al.,1994b
S-phase response, 3 weeks (200 or 16000 ppm)	↑cell proliferation	1140 mg/kg bw	Pölloth et al.,1994d
S-phase response, 3 weeks, old rats (200 or 16000 ppm)	↑cell proliferation	1140 mg/kg bw	Pölloth et al.,1994e
S-phase response, 13 weeks + reversibility (16000 ppm)	↑cell proliferation, reversible effect	1140 mg/kg bw	Mellert et al.,1996
S-phase response, 3 weeks, young rats (800 or 8000 ppm = 61 or 603 mg/kg b.w./d)	↑cell proliferation	NOAEL = 800 ppm = 61 mg/kg b.w./d. LOAEL = 8000 ppm = 603 mg/kg b.w./d	Mellert,1997
GST-P foci in liver cells (initiation assay) (2388 mg/kg)	negative (no initiating activity)	-	Gamer et al.,1995
GST-P foci in liver cells (promotion assay) (200, 800, 8000, 16000 ppm, =10.72, 42.47, 430.6 or 886 mg/kg)	positive (promoting activity) ≥800 ppm : ↑liver w ≥8000 ppm : ↑liver w, ↑GST-P$^+$ foci	NOAEL = 200 ppm = 10.72 mg/kg b.w./d. LOAEL = 800 ppm = 42.47 mg/kg b.w./d	Harada, 1997
Liver enzyme activity, rat, 3 weeks (200 or 16000 ppm, =13 or 973 mg/kg)	slight PB-like induction of hepatic enzymes	NOAEL = 200 ppm = 13 mg/kg b.w./d. LOAEL = 800 ppm = 973 mg/kg b.w./d	Mellert, 1996a
peroxisome proliferation (200 or 16000 ppm)	negative	-	Mellert et al., 1995b
examination of liver mitochondria (200 or 16000 ppm)	normal	-	Mellert et al., 1995a
induction of Cyp IA1, Cyp IIB1, CypIVA.	negative	-	Gelbke, Hildebrand, Jacob and Regenstein, meeting 21 02 1996, Brussels.
Palmitoyl Coa-oxidase	negative(no peroxisome proliferation)	-	
Liver GGT activity (200 or 16000 ppm, 3 weeks)	increased (liver necrosis)	1140 mg/kg bw	

-Kresoxim-methyl: mechanism and assessment of liver tumour induction (Van Ravenzwaay, 1996, Dossier BASF)

The studies concerning mutagenicity and genotoxicity carried out with kresoxim-methyl demonstrated the absence of a genotoxic potential indicating that the test substance was unlikely to be an initiator of carcinogenesis. This assumption was confirmed in the study in which the initiating potential was evaluated. In this study it was clearly demonstrated that kresoxim-methyl was devoid of an initiating potential.
If the test substance does not induce carcinogenesis by an initiation process, then it must be a promotor. Indirect evidence for promoting activity of kresoxim-methyl was obtained in the subchronic and chronic toxicity studies:
- An increased incidence of liver tumours was only seen at very high dose levels (≥ 400 mg/kg bw).
- The tumours were not responsible for an increased mortality in the treatment groups.
- The tumours occurred very late during the long-term studies, in fact in the high dose females which died before termination of the administration period, none were found to have liver tumours.
- The tumours were found only in the liver, which is the target organ of kresoxim-methyl toxicity.
Evidence suggestive of a tumour promotion potential can be found by looking at the liver changes induced by the test substance. These changes consisted of liver enlargement and liver weight increases (histopathologically cellular hypertrophy was noted), bile duct proliferation as well as an increase of GGT. It is of interest to note that these changes were found at dose levels which increased the liver tumour incidence, but, not at dose levels which were not carcinogenic in the rat. The fact that carcinogenicity was only noted at dose levels inducing toxicity in this organ is a common phenomenon for liver tumour promoting chemicals.

The increase of serum GGT indicates a possible cellular damage in the liver, which may result in regenerative cell proliferation. The fact that high dose administration of kresoxim-methyl does indeed result in a stimulation of cell division of hepatocytes was proven in several S-phase response studies. Liver cell proliferation was observed after short-term administration of a dose which increased liver tumours. However, no liver cell proliferation was noted at a non-carcinogenic dose level. Moreover, the increase in cell division in the liver after repeated dosing was shown to exist both in young and old rats. This latter aspect can be regarded as evidence that the test compound is able to promote initiated cells. The association of S-phase induction and liver tumour formation was demonstrated in a subchronic study. The fact that increased liver cell proliferation was still noted after 13 weeks of treatment indicates that the administration of high dose levels of kresoxim methyl results in continuous cell proliferation, a process known to be involved in tumour formation.

B.6.5.6 Summary of long-term oral toxicity and carcinogenicity (Annex IIA 5.5)

Kresoxim-methyl has an oncogenic potential at the MTD (8000 ppm) in rats and a significant increase in malignant liver tumours was reported. The primary epithelial neoplasms of the liver were cholangiomas, carcinomas, and cholangiocarcinomas. In both sexes, liver tumours occurred predominantly in animals that survived until the scheduled termination of the study.
The non-neoplastic liver lesions-eosinophilic foci of hepatocellular alteration and hypertrophy of hepatocytes-further indicate metabolic stress on the liver parenchyma. The incidence and degree of severity of minimal to severe bile duct proliferation was increased in female of high dose group(16000 ppm), while the incidence of fatty change was decreased. In male rats , significant increase in GGT was found throughout the study at the MTD.
At doses of 200 and 800 ppm, no oncogenic or systemic toxic findings were noted.

At the occasion of the resubmission, a carcinogenic study was replicated in another strain of Wistar-rats (Crl Glx Brl Han:WI) at the control and top-dose, under the assumption that the latter strain would be less sensitive to the induction of liver tumours than the initially used one (Chbb:Thom).
Liver carcinoma occurred at higher incidence in both the ♂ and the ♀ although the liver carcinoma incidence in the new study was significantly lower in the ♀, as compared to the former study.

As described above, Kresoxim-methyl has no genotoxic properties and it does not initiate the formation of liver foci. At carcinogenic doses it produced hepatic cell proliferation together with mild hepatic toxicity, both being reversible. On the basis of all data presented it can be concluded that Kresoxim-methyl is a non-genotoxic carcinogen in the rat, acting as a promotor for which a threshold dose exists.

In C57BL-strain mice, the test article was not oncogenic. Papillary necroses of the kidneys and increased number of females with liver amyloidosis associated with a higher degree of severity in females exposed to 8000ppm were considered as treatment related .

Therefore, the new results did not alter the former conclusion, being that Kresoxim-methyl was to be considered carcinogenic towards the rat (Carc. Cat. 3; Xn; R40).

Table B.6.5.6-1 : Summary of long-term oral toxicity of kresoxim-methyl

Type of test Test organism (dose ppm)	Results			purity (%) B.n°.	References
	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	critical effects		
2 yr, p.o., rat (0, 200, 800, 8000, 16000)	M :36 F: 48	M: 370 F: 503	(GGT, liver weight, (body weight, hepatocellular carcinoma	96.6%; N30 93.7%; N36 94%; N27	Mellert et al.,1994c
2 yr, p.o., rat (0, 200, 800, 8000, 16000)	M :36 F: 47	M: 375 F: 497	eosinophilic foci in liver and hepatocellular carcinoma	96.6%; N30 93.7%; N36 94%; N27	Mellert et al.,1994d
2 yr, p.o., rat (0, 16000)	-	M: 752.1 F: 1021.6	↓b.w., ↑liver w, eosinophilic and basophilic foci, spongiosis/peliosis, periportal hypertrophy in liver, hepatocellular adenoma and carcinoma	97.8%, COD-000225	Kamp et al, 2008
18 mth, p.o., mice	M: 304 F: 81	M: 1308 F: 400	↓body weight; papillary necroses (kidneys);↑number of females with amyloidosis (liver)	92.7 %; N36 95.4%; N30	Mellert et al.,1994e

B.6.6 Reproductive Toxicity (Annex II A 5.6)

B.6.6.1 Two generation reproductive toxicity in the rat (Annex II A5.6.1.)

- Report: reproduction toxicity study with Reg.n°.242 009 in Wistar rats. Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation) (Hellwig et al.,1994a, Dossier BASF)

Guidelines:
Protocol in compliance with method B of Directive 87/302/EEC.

GLP status : yes

Material and methods :
25 Wistar rats (Chbb:THOM (SPF)) /dose/sex received in the diet, kresoxim-methyl (93.7% B.n° : N 36) at doses of 0, 50, 1000, 4000 or 16000 PPM, continuously throughout the study. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals.

Measured test article intake F0 M: 5.1; 102, 411 and 1623 mg/kg bw /d
F0 F (premating): 5.6, 108, 437 and 1741 mg/kg bw/d
F1a litter: gestation period : 4.6, 91.7, 383.8 and 1482.5 mg/kg bw/d
lactation period : 8.3, 162, 661 and 2610 mg/kg bw/d
F1b litter: gestation period : 4.3, 84.3, 349.8 and 1389 mg/kg bw/d
lactation period : 7.1, 143.2, 587.2 and 2409 mg/kg bw/d

Findings in the adults:
The main treatment related effects are given in the table B.6.6.1-1. It appears that the MTD was reached.
There were no treatment related effects on survival, clinical signs, mating behavior and fertility. Mating index was 100% in all groups and the mean number of F1a and F1b pups delivered/dam did not show biological differences between groups. Decreases in body weight were related with treatment from the dose of 4000 ppm on.

Table B.6.6.1-1 : Two generation reproductive toxicity in the rat - adult data

Endpoint/dose	0		5 0 ppm		1000 ppm		4000 ppm		16000 ppm	
	M	F	M	F	M	F	M	F	M	F
Adult rat F0										
food consumption						↘wk 2,3,4,5		↘wk 0,1	↘wk 1; 3%	↘wk1,2,3, 4; 5%
body weight						↘ wk 3,4,5,6	↘wk 4→23	↘ wk 2,3,4,5	↘wk 2↘29;8%	↘wk 3,4
body weight gain					↘		↘8%	↘10%	↘11%	↘10%
clinical chemistry										
AP					↘	↘	↘	↘	↘	↘
ALT					↘		↘		↘	↘
GGT							↗		↗	
histopatology										
liver fat storing cell number							↘		↘	
Adult rat F1										
food consump.									↘	↘
body weight							↘wk 5→end	↘wk 1,3,14	↘10%	↘9%
body weight gain							↘		↘	↘
clinical chemistry										
AP					↘	↘	↘	↘	↘	↘
ALT							↘	↘	↘	↘
GGT									↗	↗
kidney weight								↘(a)		↘(a)
histopathology: liver fat storing cell number							↘		↘	
mating index (%)	96		88		96		100		92	
Statistically significant increase (↗)or decrease (↘) (a):absolute										

Comments with regard to the adults:
The decreases in AP and ALT did not show a dose-response relationship and were , most probably, due to a slight alteration in food resorption. The increased GGT and a decreased number of fat storing cells in the liver in males point to the liver as target organ, without, however, an impairment of liver function or of histopathological lesions.

Findings concerning the litters:
The main treatment related effects are given in the table B.6.6.1-2 It appears that the MTD was reached.

Table B.6.6.1-2 : Two generation reproductive toxicity in the rat-litter data.

Endpoint/dose	0	5 0 ppm	1000 ppm	4000 ppm	16000 ppm
Litter data F1:					
body weight				↘F1a; F1b	↘F1a; F1b
clinical observations		filiformed tail, traumatic lesion of hindlimb, microphtalmia, chromodacryorrhea, unsteady gait, hydrocephalus			
retarded growth				↗	↗
fertility rate (%):					
F1a litter	100	92	100	88	96
F1b litter	100	96	100	92	96
F2 generation pups/litters:					
clinical signs		no clear dose-response effect			
b.w./b.w. gain				↘	↘

statistically significant increase (↗)or decrease (↘) ($p<0.05$)

Other findings:
There were no treatment related effects for the F1a and F1b pups with regard to percentages of liveborn and stillborn pups, pup mortality/viability during the lactation periods, sex distribution and sex ratios of live pups on the day of birth and on day 21 p.p.
Retarded morphological development was observed in the 2 high doses (delays in pinna unfolding) in the F1b pups. Behavioural tests were normal. Only some of the F1a and F1b pups showed findings at necropsy. for example, post mortem autolysis, sloped incisors, cleft palate, hernia diaphragmatica, dilated renal pelvis, hydroureter, traumatic hindlimb lesion, malformation of tail or head, microphtalmia, hydrocephalus, agnathia and micrognathia occurred in several pups . All necropsy findings, however, noted for the F1a and/or F1b pups do not show a clear relation to dosing and /or can be found at a comparable incidence in the historical control data.
Food consumption of F1 generation males and females of the 16000 ppm was diminished and considered to be substance-related.
The impairments in body weights/weight gains recorded for the 4000 and/or 16000 ppm F1 parental animals have to be associated with the test substance administration.
No clinical signs which might have been attributed to the test substance administered were detected in male or female F1 generation parental animals.
The male and female mating index reached 100% in all groups. The differences concerning fertility index between the groups are finally assessed as spontaneous in nature. There were no biologically relevant differences between
test groups and controls concerning the mean duration of gestation and the number of liveborn and stillborn F2 pups. All pregnant females gave birth to litters with liveborn pups.
AP and ALT activities decreased but there was no clear dose-response relationship . At the highest dose, significantly increase in serum GGT was observed in both sexes.
F2 generation:
Pup number of delivered F2 pups/dams, pup viability/mortality and sex ratio differences are in the range of biological variation.
Mean body weight/body weight gains of the F2 male and female pups of the 16000 ppm were clearly influenced by the test substance. These effects, less pronounced were also present at 4000 ppm. All the clinical observations are regarded as spontaneous in nature. All differences in development stages and behavioural tests are without biological relevance. The examination of F2 pups at necropsy did not reveal any differences considered to be of biological relevance.
Comments and conclusion :
kresoxim-methyl had no adverse effects on reproductive parameters of the parental animals of either generation (F0 and F1) of all groups. Clear signs of developmental toxicity were noted for the progeny of the high dose of the F0 and F1. In all three pup generations (F1a, F1b and F2) impaired body weight/body weight gain and some indications for delays in the morphological development (F1b pups only) were seen. Similar, but much less

pronounced effects were also observed for the F1a, F1b and F2 pups at 4000 ppm. The 2 low doses were tolerated by the offspring's of both parental generations without any changes which could be causally related to test substance administration.
NOAELreproductive function = 16000 ppm = 1.5 g/kg bw/d
NOAELsystemic tox. = 1000 ppm= 100 mg/kg bw/d

B.6.6.2.1 Teratogenicity test by the oral route in the rat (Annex IIA 5.6.2)

- Report: study of the prenatal toxicity of Reg.n°.242 009 in Wistar rats after oral administration (gavage) (Hellwig and Hildebrand, 1994, Dossier BASF)
Guidelines:
Protocol in compliance with the method B of Directive 87/302/EEC .
GLP status : yes
Material and methods :23- 25 pregnant Wistar rats/dose (Chbb:THOM (SPF)) received by gavage, an aqueous suspension of kresoxim-methyl (93.7% B.n° : N 36) at doses of 100, 400 and 1000 mg/kg bw on day 6 through day 15 post coitus. Controls were dosed with vehicle only (0.5% aqueous CMC). Females were sacrificed on day 20 post coitus.
Findings :
Dams :
Body weights, body weight gains and food consumption of the female rats did not show any differences of biological relevance if compared to the controls. There were no abnormal clinical findings nor mortalities in any of the groups. There were no substance-related differences between the groups in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the postimplantation losses, the number of resorptions and viable fetuses (table B.6.6.2.1-1).

Litters:
- *offspring* : the sex distribution of the fetuses was comparable with the control fetuses. The mean placental weights and the mean fetal body weights were not influenced by the test substance administration.
- *abnormalities :*
The external examination revealed 3 fetuses with external malformations.
In the high dose group, a dead fetus was recorded from a mother with a placenta necrobiotic. This effect was not associated with the treatment.
2. The examination of the organs of the fetuses revealed one soft tissue malformation : hydrocephaly with dilatation of both lateral brain ventricles. Hydrocephaly occurs also spontaneously in control fetuses of the rat strain used.
All these malformations are also present at a low incidence in the historical control data.
3. Skeletal malformations of the sternum, the ribs and/or the vertebral column were seen without any statistically
significant differences between the substance-treated groups and the control group.
The only statistically significant difference between the substance-treated groups and the control group concerning skeletal retardations was an incresed occurrence of incompletely ossified thoracic vertebral body/bodies in the 1000 mg/kg bw but these values were fully within the historical control range and were therefore, considered to be spontaneous in nature.
The differences observed between the control and the substance-treated groups appeared either without a clear dose-response relationship and/or were assessed to be without biological relevance, because the relevant values/findings are to be found in a similar or even higher range/incidence within the historical control data (table B.6.6.2.1-2).

Table B.6.6.2.1-1 : Teratogenicity test by the oral route in the rat - Dams data

Endpoint/dose	0	100 mg/kg bw/d	400 mg/kg bw/d	1000 mg/kg bw/d
dams data: clinical signs:		no clinical signs		
mortality		no mortality		
food consumption		no effect		
body weight:		no effect		

Table B.6.6.2.1-2 Teratogenicity test by the oral route in the rat - Fetal data

Endpoint/dose	0	100 mg/kg bw/d	400 mg/kg bw/d	1000 mg/kg bw/d
Foetal observations mortality				1
body weight		no effect		
sex distribution		no effect		
placenta weight		no effect		
external malformation (n)		Anasarca +cleft palate(1)	acaudia (1)	Meningocele +microphtalmia(1)
soft tissue malformed.(n)				Hydrocephaly +dilatation of late ral brain ventricles (1)
skeletal malformed.:				
sternum, ribs,vertebral column	5/168	11/167	13/156	10/172

(n): number fetuses affected

Conclusion :
No substance related adverse effects were observed in dams and there were no indications of embryo-/fetotoxicity and especially no substance-induced signs of teratogenicity in this study.
NOAEL maternal toxicity = NOAEL embryotoxicity = 1000 mg/kg bw/d

B.6.6.2.2 Teratogenicity test by the oral route in the rabbit (Annex IIA 5.6.2)

- Report: study of the prenatal toxicity of Reg.n°.242 009 in rabbits after oral administration (gavage) (Hellwig and Hildebrand, 1994b, Dossier BASF)
Guidelines:
Protocol in compliance with method B of the Directive 87/302/EEC.
GLP status : yes
Material and methods :13- 15 pregnant Himalayan rabbits/dose (Chbb: HM (outbred)) received by gavage, an aqueous suspension of kresoxim-methyl (96.6% B.n° : N 30) at doses of 100, 400 and 1000 mg/kg bw on day 7 through day 19 post insemination. Controls were dosed with vehicle only (0.5% aqueous CMC). Females were sacrificed on day 29 post insemination.
Findings :
Dams :
Food consumption, body weight and body weight changes were uninfluenced by the test substance administration.
Uterus weights were not affected by exposure.

There were no substance-related and/or statistically significant differences in conception rate, mean number of corpora lutea and implantation sites or in the values calculated for the pre- and postimplantation losses, number of resorptions and viable fetuses.
Litters :
Sex distribution of fetuses, weight of placentae and weight of fetuses were not influenced by the oral administration of the test substance.
External examination of foetuses : microcephaly and brachygnathia were observed in 1 low dose foetus. One type of external variation (pseudoankylosis) was seen (1 of control, 4 of 400 and 1 of 1000 mg/kg bw).
Several types of soft tissue malformations were observed in fetuses of does from the substance treated groups, but not from the control group: septal defect, gallbladder agensia, hydrocephalus, aortic arch and aorta descendens dilatation. Variations were detected in each group including control : heart with traces of interventricular foramen/septum membranaceum; dilated renal pelvis and hypoplasia of gallbladder.
Skeletal examination: no malformations were observed in the control and high dose group. Malformations of the foetal skeletons (vertebral column, fused thoracic vertebrae, sternum and limbs)were noted for 2/81 low dose foetuses and 2/106 intermediate dose. Variations were related to the skull, the ribs, the vertebral column and the sternum.
In all groups signs of retardation's were found (incomplete or missing ossification of skull bones, vertebral column, sternebrae and talus).

There are no statistically significant differences of biological relevance between the control and the substance treated groups with respect to external, soft tissue, skeletal or total malformations, variations and / or retardation's .
Conclusion:
The administration of kresoxim-methyl to pregnant Himalayan rabbits induced no teratogenic effects up to and including the dose of 1000 mg/kg bw/d.
NOAEL maternal toxicity : 1000 mg/kg/d
NOAEL embryotoxicity and teratogenicity : 1000 mg/kg/d

B.6.6.3 : Summary of reproductive toxicity and teratogenicity (Annex IIA 5.6.2)

Table B.6.6.3-1 :Summary of reproductive toxicity and teratogenicity of kresoxim-methyl

Type of test Test organism	Results			kresoxim-methyl, purity (%) batch number	References
	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	critical effects		
2 gen., rat, p.o.	100	424	F0: ↘body weight; ↗ serum GGT; ↘ liver fat storing cells F1b pup: retarded morpho.devel.	93.7% ; N 36	Hellwig et al.,1994a
terato., rat, p.o.	1000 (maternal +fetal)	-	no effect	93.7% ; N 36	Hellwig and Hildebrand,1994
terato., rabbit, p.o.	1000 (maternal +fetal)	-	no effect	96.6% ; N 30	Hellwig and Hildebrand,1994a

Kresoxim-methyl had no adverse effects on reproductive parameters of rats , but produced some dose - dependent developmental toxicity in the pups, impaired body weight/body weight gain and some indications for delays in the morphological development at doses which produced clear parental toxicity. The overall and developmental NOAEL were 100 and 1500 mg/kg bw/d, respectively.
No substance related adverse effects were observed in pregnant rats and Himalayan rabbits and there were no indications of embryo-/fetotoxicity and especially no substance-induced signs of teratogenicity .

B.6.7 Delayed neurotoxicity (Annex II A 5.7)

No data, not necessary.

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicity studies on metabolites (Annex IIA 5.8.1)

No data, not necessary.

B.6.8.2 Additional studies (Annex IIA 5.8.2)

B.6.8.2.1 Study on the influence of lowered esterase activities on the toxicological profile of Kresoxim-methyl.

This study was performed to assess whether inhibition of esterases that detoxify Kresoxim-methyl by cholinesterase inhibitors may potentiate the toxicity of Kresoxim-methyl.

- **Report :Kresoxim-methyl (Reg.n° 242009) Pilot study : dietary administration to rats after repeated pretreatment with Dimethoate (Mellert et al.,1996a; Dossier BASF)**

Material, methods and findings:
3 Wistar rats/sex (main group) or 4 Sprague-Dawley female rats (satellite groups) received by gavage Dimethoate at 40 mg/kg bw/d for 3 days. From the same day onwards the animals of the main groups were treated with kresoxim-methyl (Reg.n°.242 009; B.n°. N36; 94.3%) at dietary concentrations of 8000 and 800 ppm for another 6 days.

Treatment with Dimethoate for 3 days resulted in reduced serum cholinesterase activities by about 67%, and in reduced erythocyte cholinesterase activies by about 78%. After 3 days of recovery, cholinesterase activities of the satellite groups were still inhibited by about 32% in the serum and by about 49% in the erythrocytes.No additional clinical signs of toxicity were observed during treatment with Kresoxim-methyl. Clinical pathology at the end of the study did not show any substance-related findings, besides lowered cholinesterase activities in erythrocytes in all groups pretreated with Dimethoate. Liver weights did not show any treatment-related effects in any of the test groups.

Repeated pretreatment of rats with Dimethoate did not enhance the toxicity of Kresoxim-methyl (Reg.n°.242 009) under the conditions of this study. Substantially lowered esterase activities have therefore no influence on the toxicological profile of Kresoxim-mehtyl.

Conclusion:
Repeated pretreatment of rats with dimethoate did not enhance the toxicity of kresoxim-methyl under the conditions of this study. Substantially lowered cholinesterase activities in serum or erythrocytes have therefore no influence on the toxicological profile of kresoxim-methyl.

B.6.8.2.2 Effects of kresoxim-methyl on the vital functions of animals

- Effects on the vital functions of animals- general pharmacology of- Reg.n°.242 009 (Dubach-Powell et al.,1994, Dossier BASF)

1. General behaviour of mice:
Material, methods and findings:
3 male NMRI mice outbred, SPF/dose received 1 dose of kresoxim-methyl (B.n°.N36, 93.7%) at 0, 1000, 2000, 5000 mg/kg bw by gavage.

The acute effects of kresoxim-methyl on the behaviour was investigated:

5000 mg/kg bw:2/3 mice showed a slight change in their body carriage i.e. hunched posture. However, this finding was also seen in1/3 mice at 1 h after exposure to 2000 mg/kg bw and in 1/3 mice treated with 1000 mg/kg bw at 2 h after dosing.
In the control group, 2/3 animals receiving vehicle (carboxymethyl cellulose) only were also recorded to have a hunched posture at 2 h after treatment.
Observations were attributed to the administration of the vehicle.

2. Effects on spontaneous locomotor activity in mice:
Material, methods and findings:
4 maleNMRI mice outbred, SPF/dose received 1 dose of kresoxim-methyl,(B.n°.N36, 93.7%) at 0, 1000, 2000 or 5000 mg/kg bw by gavage.
The acute effects of a single oral administration of kresoxim-methyl on the locomotor activity were recorded at 1/2,1, 2, 4 and 8 h after treatment.
All types of activity decreased after administration of both the vehicle and the test article. These effects were most severe between 2 and 4 h post dosing with evidence of a partial or complete recovery by 8 h after administration.
Observations were attributed to the administration of the vehicle.

3. Effects on intestinal motility in mice:
Material, methods and findings:
6 male NMRI mice outbred, SPF/dose received 1 dose of kresoxim-methyl,(B.n°.N36, 93.7%) at 0, 1000, 2000 or 5000 mg/kg bw by gavage.
A single oral administration of kresoxim-methyl at the tested doses had no effect on the gastro-intestinal motility of mice.

4. Effects on cardiovascular and respiratory systems of the rat:
Material, methods and findings:
4 male outbred Wistar rats/dose received 1 dose of kresoxim-methyl (B.n°.N36, 93.7%) at 0 or 5000 mg/kg bw intraduodenally.
Systolic, diastolic and mean blood pressure, heart rate, respiratory rate, tidal volume and minute volume were directly recorded and continuously computed at 5 min intervals starting 15 min before dosing and for up to 60 min after dosing.
Neither the vehicle alone, nor 5000 mg/kg bw compound in vehicle, were found to have any major effects on either the cardiovascular or respiratory parameters.

5. Effects on neuromuscular junction: gastrocnemius muscle preparation in the rat:
Material, methods and findings:
4 male outbred Han Wistar rats/dose were used; they received 1 dose of kresoxim-methyl (B.n°.N36, 93.7%) at 0 or 5000 mg/kg bw intraduodenally.
After induction of anesthesia, the right Achilles tendon was fixed to a force displacement transducer. Left carotid artery was cannulated and connected to a pressure transducer. A cannula was introduced into the duodenum and the animals received 1 dose of kresoxim-methyl (B.n°.N36, 93.7%) at 0 or 5000 mg/kg bw . The sciatic nerve was cleared of connecting tissue and fixed in a bipolar electrode.
The group mean amplitude of muscle contraction was constant throughout the 60 minute recording period after treatment with both vehicle and kresoxim-methyl at 5000 mg/kg bw. Blood pressure and heart rate were unaffected.

6. Effects on the renal function and hepatic system of the rat:
Material, methods and findings:
6 male outbred Wistar rats/dose received 1 dose of kresoxim-methyl (B.n°.N36, 93.7%) at 0, 1000, 2000 or 5000 mg/kg bw by gavage and were deprived of food for a period of 24 h. Blood was collected at 24 h after exposure and urine were sampled 8 and 24 h after treatment. Urinalysis and clinical biochemistry were performed on the different samples.

The assessment of urinalysis data 8 and 24 h after dosing indicated a slight decrease in the urine output by 35 to 42% at 8 h and a slight increase by 63% in the AP, 24 h after dosing with 5000 mg/kg bw. The results of clinical biochemistry investigations 24 h after dosing show a reduction of ALT activity in all exposed groups without alteration of AP and AST.
Pathological investigations revealed that no treatment-related morphological alterations were induced in the kidney and liver .

7. Effects on the isolated ileum of the guinea pig:
Material, methods and findings:
2 male Ibm: GOHI albino guinea-pig were used for ileum preparation. Four ileum strips were tested simultaneously in the presence of histamine or acetylcholine. kresoxim-methyl (B.n°.N36, 93.7%) was added at a concentration of 10^{-7}, 10^{-6} and 10^{-5} g/ml in the organ bath.
In this assay, kresoxim-methyl showed non-competitive antagonistic activity against the contractions induced by both histamine, a histamine$_1$ receptor agonist in this tissue , and acetylcholine, a muscarinic receptor agonist. Effects were only apparent at and above 10^{-6} g/ml and all effects appeared to be fully reversible.
kresoxim-methyl possess weak, reversible, antagonistic activity to smooth muscle contractions.

8. Effects on the isolated trachea of the guinea pig:
Material, methods and findings:
2 male Ibm: GOHI albino guinea-pig were used . After cervical dislocation, the excised trachea was fixed in the organ bath and pre-stretched. Four strips of trachea were tested simultaneously in the presence of histamine or acetylcholine. kresoxim-methyl (B.n°.N36, 93.7%) was added at a concentration of 10^{-7}, 10^{-6} , 10^{-5} or 10^{-4} g/ml in the organ bath.
In this assay, kresoxim-methyl showed non-competitive antagonistic activity against the contractions induced by both histamine, a histamine$_1$ receptor agonist in this tissue , and acetylcholine, a muscarinic receptor agonist. Effects were only apparent at and above 10^{-5} g/ml and all effects appeared to be fully reversible.
kresoxim-methyl possess weak, reversible, antagonistic activity to smooth muscle contractions.

9. Effects on *in vitro* hemolysis:
Material, methods and findings:
2 male outbred Han Wistar rats were used. After light anesthesia, blood was sampled from the retro-orbital plexus. A suspension of erythrocytes was prepared and kresoxim-methyl (B.n°.N36, 93.7%) was added at a concentration of 1, 10 or 100 µg/ml. Two hours after incubation at 37°C, tubes were centrifugated and absorbance was measured in the supernatant.
kresoxim-methyl was found to have no effect on hemolysis *in vitro*.

10. Effects on the spontaneous electroencephalogram of the rat :
Material, methods and findings:
4 male outbred Han Wistar rats were used. After anesthesia, the skull was exposed and single electrodes were implanted above the frontal and the occipital cortex together with an indifferent electrode above the cerebellum and an electrode in the neck muscle.The first recordings were carried out at the earliest 10 days after the electrode implantation. Kresoxim-methyl (B.n°.N36, 93.7%) was administered via a stomach tube and each animal received in successive order: the vehicle (CMC), then 1000, 2000 and 5000 mg/kg bw with a 3-9 d washout period between each administration.
At 1000 and 5000 mg/kg, the power of the frontal cortex was decreased. With respect to the changes of vigilance stages the no effect dose was 1000 mg/kg. AT the 2 higher doses a prolongation of classical sleep I , the light sleep, accompanied by a shortening of SWS and PS-spindles was observed.
At 5000 mg/kg sleep latency was shortened and waking was decreased during the first 3 recording hours followed by a rebound during the following 3 h suggesting that the initial sedation was probably induced by a peripheral effect and not by a direct effect of the compound on the brain. The use of a 5% CMC solution did not change the CNS-activity. There were no quantitative differences between Waking and PS after the administration of 1000 and 2000 mg/kg. The significant shortening of SWS and PS-spindles correlates to the prolongation of the light sleep.
Kresoxim-methyl did not induce abnormal EEG-phenomena or behaviour.

Kresoxim-methyl substantially altered the vigilance stages as well as the power of the EEG only at the highest dose of 5000 mg/kg. This EEG-study did not indicate an acute CNS intoxication by kresoxim-methyl.

B.6.8.3. Neurotoxicity studies

B.6.8.3.1. Acute oral neurotoxicity studies

Study:Reg. No. 242009-Acute oral neurotoxicity study in Wistar rats.

-Rat, Kresoxim-Methyl, 0; 500; 1000 or 2000 mg/kg bw, acute oral, main study (Mellert, 1996c)

Findings:

Mortality: none

Clinical signs:	no relevant finding.
Body weight:	no relevant finding.
Functional Observational Battery (FOB):	no relevant finding.
Home-cage observations:	no relevant finding.
Open-field observations:	no relevant finding.
Sensimotor tests-reflexes:	no relevant finding.

Quantitative observations (grip strength, landing foot splay):
On d14, significantly reduced forelimb strength was measured in the ♀ treated at 1000 mg/kg b.w (3.0±0.4) and 2000 mg/kg b.w. (2.9±0.6), when compared to the study control (3.4±0.3). According to the notifier, the magnitude of the decrease compared to the study control was quite low (12-15%). In addition, the historical control range was stated 3.3±0.7, which means that a range of 2.6-4.0 could be considered a 'normal range' (in the notifier's report, a m±2×s.d. range is considered). The HCD data were obtained in 19 studies, with n=90 rats, time unknown.
Although a light dose-response was visible, the effect is only reported in the ♀, and at the last time-point (not on d0 or d7). Hindlimb strength remained unaffected at any time-point, and similar effects were not observed in a repeated 90d neurotoxicity protocol. Other neurotoxicity parameters were also unremarkable.

Motor activity measurements:
Both overall or single activity was increased in mid- and high-dose ♂ or high-dose ♀. However, dose-response relationship was absent in any case, and d -6 ♂ (pre-test) also showed significantly higher values when compared with controls. Therefore, the findings were considered furtuitous and without toxicological relevance.

Table B.6.8.3.1-21:
acute neurotoxicity study of Kresoxim-Methyl in rats: forelimb grip strength (Mellert, 1996c):

Dose (mg/kg)	**0**		**500**		**1000**		**2000**	
	♂	♀	♂	♀	♂	♀	♂	♀
d -6	2.7	3.4	2.3	3.1	2.7	2.8	2.8	3.2
d 0	3.2	3.4	3.0	3.3	3.4	3.5	3.3	3.4
d 7	3.8	3.3	3.8	3.2	3.9	3.2	3.8	3.1
d 14	3.9	3.4	3.6	3.3	3.9	3.0*	3.8	2.9**

Values expressed as means (Newton); Statistically significant modification: *p≤0.05 and **p≤0.01 (Mann-Whitney U-test)

Gross necropsy and histopathology:
A spontaneous single proximal sciatic nerve degeneration of minimal degree was observed in one top-dose ♀. As no other nervous tissue was affected, and other animals of this dose-groups showed no axonal degeneration, this was considered a chance event.

Conclusions:

Neuro(toxicity) NOAEL = 2000 mg/kg
Neuro(toxicity) LOAEL > 2000 mg/kg

Guidelines: No EC protocol is available
Protocol partly in compliance with test method OPPTS 870.6200 of US EPA
Deviation:
The motor activity data are hardly interpretable, since pre-test (d-6) measurements in mid- and high dose ♂, and high-dose ♀ were significantly increased, precluding meaningful conclusions on this parameter 0d, 7d or 14d post-treatment.

GLP status: The study is GLP.
Materials and methods:
Ten rats/sex/dose (Wistar Chbb: Thom (SPF)) received Kresoxim-Methyl (93.7%; B.nr. N36) dispersed in 0.5% carboxymethylcellulose in water by gavage at a dose level of 0; 500; 1000 or 2000 mg/kg bw (dose volume 10 mL/kg). Dose levels were 101-108% of nominal values. Compound stability for 1 week in the refrigerator, and compound homogeneity were stated to be satisfactory. Besides the clinical and mortality observations (daily) and body weight measurements (weekly), animals were tested for the Functional Observation Battery (FOB) endpoints and motor/locomotor activity on d-6, d0 (h2-h6), d7 and d14. FOB included following observations: cage-side behaviour, behaviour during handling, open field behaviour and reflex/physiologic endpoints. Motor (no locomotor) activity was studied by assessing rat behaviour in a cage, equipped by 4 IR emittor/detector pairs. The movements were automatically sampled in 5-minute intervals (total test session: 90 minutes). Motor activity is defined as the number of beam interruptions/session.
All animals were subjected to gross necropsy, and 5 rats/sex/dose were chosen at random for perfusion and tissue collection. In control- and top-dose animals, subsequent histopathologic evaluation of selected tissues, including brain, gasserian and dorsal root ganglia, spinal chord, peripheral nerve tissue (sciatic, tibial, sural) and gastrocnemius muscle was performed. Sensitivity, reliability and validity of the test procedures have been established in *previous* studies with acrylamide, carbaryl, 3,3-iminodiproprionitrile, nomifensin and diazepam.
Rats selected for histopathology were sacrificed by perfusion fixation, while all others were sacrificed by CO_2 asphyxiation.
In a *preliminary range-finding study*, 5 rats/sex were dosed at 5000 mg/kg bw. No deaths occurred and no clinical signs were observed. From these data, no peak of neurobehavioral effects was established and it was decided to conduct FOB and motor activity measurements at 2h and 6h following treatment respectively.
The study is accepted.

B.6.8.3.2 Subchronic oral neurotoxicity study

-Rat, Kresoxim-Methyl in diet, 0; 1000; 4000 or 16000 ppm for 90d, (Mellert, 1996d)

Findings :
Mortality: none
Clinical signs: no relevant findings.

Food consumption:
was slightly impaired in the top-dose ♂, attaining statistical significance on d7 (-8%, p≤0.05). In the top-dose ♀, a 7-13% decrease was measured, attaining statistical significance occasionaly.

Body weight:
was decreased by about 9% at the top-dose. Statistical significance was attained on d84 (♂) and on several days in the ♀. On these days, body weight gain was also decreased by 17%(♂)-32%(♀).

Functional Observational Battery (FOB): no relevant findings.

Grip strength: no relevant findings; occasional excursions from control values in the low- and mid-dose ♀ were considered toxicologically irrelevant, in the absence of effects at the higher doses.

Foot Splay: no relevant findings; occasional excursions from control values in the mid-dose ♀ were considered toxicologically irrelevant, in the absence of effects at the top-dose.

Motor activity measurements:

Motor activity was increased sporadically at some sampling intervals in the treated ♂ animals. However the findings were in the absence of any dose-response relationship, and at pre-dosing (d–7) ♂ assigned to mid- and high dose groups also showed significantly higher values when compared with controls.
Non dose-dependent, and for this reason irrelevant excursions from control values were also noted in the treated ♀ animals. Therefore, the findings were considered furtuitous and without toxicological relevance.

Gross necropsy and histopathology: no relevant findings; a minimal axonal degeneration in the sural nerve of one top-dose ♂ was considered furtuitous, in the light of several other degeneration findings in the control groups.

Conclusions:
Kresoxim-Methyl was considered devoid of neurotoxicological potential under the conditions of this study.

toxicity NOAEL	=4000 ppm	=292 mg/kg bw/d
toxicity LOAEL	=16000 ppm	=1180 mg/kg bw/d
	(based upon ↓feed consumption, ↓body weight (gain)	

neurotoxicity NOAEL	=16000 ppm	=1180 mg/kg bw/d
neurotoxicity NOAEL	≥16000 ppm	≥1180 mg/kg bw/d

Guidelines: No EC protocol is available
Protocol partly in compliance with test method OPPTS 870.6200 of US EPA
Deviation:
The motor activity data are hardly interpretable, since pre-test (d-7) measurements in mid- and high dose ♂ were significantly increased, precluding meaningful conclusions on this parameter 22d, 50d or 85d post-treatment.

GLP status: The study is GLP.
Materials and methods:
10 rats/sex/dose (Wistar Chbb: Thom (SPF)) were fed a diet of Kresoxim-Methyl (93.7%; B.n° N36) at a dose level of 0; 1000; 4000 or 16000 ppm during 90 days. Samples were checked analytically to ensure accuracy, homogenicity and stability of the administered diet. Dose levels were 94.8-101.8% of nominal values. Compound stability for 32 days at ambient temperature, and compound homogeneity were satisfactory. Achieved doses (taking into account diet analysis results): ♂: 0; 72; 292 and 1180 mg/kg b.w. and ♀: 0; 84; 341 and 1354 mg/kg b.w. Besides the clinical and mortality observations and body weight measurements, animals were tested for the Functional Observation Battery (FOB) endpoints and motor activity on d-7, d22, d50 and d85. Ophthalmologic examination in order to exclude animals with possible visual failure were not conducted. All animals were subjected to gross necropsy, and 5 rats/sex/dose were chosen at random for perfusion and tissue collection. In control- and top-dose animals, subsequent histopathologic evaluation of selected tissues as described previously (Mellert, 1996c). The top dose was selected taking into account the effects observed in a previous subchronic toxicity study (Mellert et al., 1994d).
The study is accepted.

B.6.8.4 Toxicological studies on metabolites

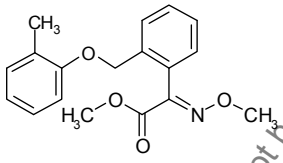
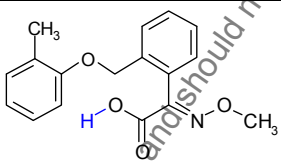
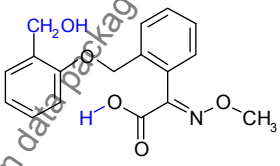
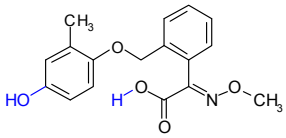
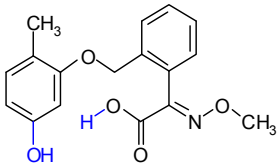
Both acute toxicity and bacterial mutagenicity studies were conducted on plant, animal or soil metabolites Reg N° 262 451, Reg. N° 291 685, Reg. N°292 932 and on Reg. N°339 774.

Following studies were conducted:

	Code Reg. N.°	origin of metabolite*	Studies	
			Acute oral rat	Bacterial genotoxicity
1	**262 451**	**r (3.9% U – 7.1% F),** g, h, w, a, gr, sb, rc, h	x	x
2	**291 685**	**r (6.5% U – 5.8% F),** g, w, gr	x	x
3	**292 932**	**r (15.8% U - 13.3% F),** g, h, w, a, gr, rc	x	x
4	**339 774**	gr	-	x

*: r: rat (U: urine, F: faeces), g: goat; h: hen; w: wheat; a: apple; gr: grape; sb: sugar beat; rc: confined rotational crop; h: hydrolysis

Codes, name and structure of tested metabolites:

	Code	Structure	Chemical name
	Reg. N°. 242 009		Kresoxim-Methyl methyl 2-[o-(o-methylphenoxymethyl) phenyl]-2-(methoxyimino) acetate
1	Reg. N.° 262 451 BF 490-1		(E)-2-methoxyimino-2-[2-(o-tolyloxymethyl)phenyl]acetic acid
2	Reg. N.° 291 685 BF 490-2		2-[2(2-hydroxymethylphenoxymethyl)phenyl]-2-methoxyiminoacetic acid)
3	Reg. N.° 292 932 BF 490-9		2-[2-(4-hydroxy-2-methylphenoxymethyl)phenyl]-2-methoxyiminoacetic acid
4	Reg. N.° 339 774 BF 490-15		2-[2-(5-hydroxy-2-methylphenoxymethyl)phenyl]-2-methoxyiminoacetic acid

B.6.8.4.1 Acute oral toxicity studies on the metabolites

B.6.8.4.1.1 Rat, Reg. n° 262 451, oral, 200, 2000, 5000 mg/kg bw, LD_{50}-test (Kirsch, 1995)

Report: Study on the acute oral toxicity of Reg.No. 262 451 in rats; [redacted] 1995/10213

Findings:
Mortality:

	♂		♀		♂+♀
Dose (mg/kg b.w.)	**mortality**	**day of death and number ( )**	**mortality**	**day of death and number ( )**	**mortality**
200	0/5	-	0/5	-	-
2000	2/5	d1(0); d1(1)	4/5	d0(4)	6/10
5000	5/5	d0(5)	3/5	d0(5)	10/10

Clinical signs:
At 2000 and 5000 mg/kg b.w., poor general state, staggering gait, dyspnoe, apathy and piloerection were observed in most animals. In addition, top-dose animals exhibited lateral position, atonia and paresis occasionaly.
At 2000 mg/kg b.w., impaired general state, abdominal position, tremor, twitching, erythema and lachrimation were observed. Most signs were restricted on the day of dosing, except piloerection, which was observed up to and including d5.
At 200 mg/kg b.w., clinical signs were unremarkable.

Body weight: No abnormalities detected.

Necropsy :
Decedents showed discolored (white) and watery content in stomach and intestines.
No abnormalities were detected in the survivors.

Conclusions:
LD_{50} (♂)~2000 mg/ kg b.w.
LD_{50} (♀)~1090 mg/ kg b.w.
LD_{50} (combined) ~2000 mg/kg bw.
Metabolite Reg. N° 262 451 is considered harmful by oral uptake in the rat (Xn; R22)

Guidelines: Protocol in compliance with test method B.1 of directive 92/69/EEC
GLP status: The study is GLP.
Materials and methods:
5 rats/sex (Wistar, Chbb: Thom(SPF)) received Reg N° 262 451 (purity not reported; B.n° 00689-145) suspended in 0.5% Tylose (Na Carboxymethylcellulose) by gavage, at a dose level of 200, 2000 or 5000 mg/kg b.w. (dose volume 20 mL/kg).
No *preliminary experiment*, the doses were selected on the basis of experimental results of acute toxicity studies on similar substances.The study is accepted.

B.6.8.4.1.2 Rat, Reg. n° 291 685, oral, 5000 mg/kg bw, limit-test (Kirsch, 1994b)

Report: Study on the acute oral toxicity of Reg.No. 291 685 in rats [redacted] 1994/11472

Findings:
*Mortality:*none
Clinical signs: no relevant findings.
Body weight: no relevant findings.

Necropsy :

No abnormalities were detected in the survivors.

Conclusions:
LD_{50} (♂) >5000 mg/ kg b.w.
LD_{50} (♀) >5000 mg/ kg b.w.
LD_{50} (combined) >5000 mg/kg bw.
Metabolite Reg. N° 291 685 is not considered harmful by oral uptake in the rat.

Guidelines: Protocol in compliance with test method B.1 of directive 92/69/EEC
GLP status: The study is GLP.
Materials and methods:
5 rats/sex (Wistar, Chbb: Thom (SPF)) received Reg N° 291 685 (purity not reported; B.n° 00436-65) suspended in 0.5% Tylose (Na Carboxymethylcellulose) by gavage, at the limit dose level of 5000 mg/kg b.w. (dose volume 20 mL/kg).
No *preliminary experiment*, the dose was selected on the basis of experimental results of acute toxicity studies on similar substances.The study is accepted.

B.6.8.4.1.3 Rat, Reg. n° 292 932, oral, 2000, 3000, 5000 mg/kg bw, LD_{50}-test (Kirsch, 1994a)

Report: Study on the acute oral toxicity of Reg.No. 292 932 in rats: [redacted] 1994/11171

Findings:
Mortality:

	♂		♀		♂+♀
Dose (mg/kg b.w.)	**mortality**	**day of death and number ( )**	**mortality**	**day of death and number ( )**	**mortality**
2000	0/5	-	0/5	-	0/10
3000	0/5	-	0/5	-	0/10
5000	0/5	-	1/5	d1(1)	1/10

Clinical signs:
At 5000 mg/kg b.w., impaired/poor general state, dyspnoe, and piloerection were observed in most animals. The animals treated at 2000 or 3000 mg/kg b.w. showed no clinical signs.

Body weight: No abnormalities detected.

Necropsy :
Decedent showed dicolored (white) and watery content in stomach and intestines.
No abnormalities were detected in the survivors.

Conclusions:
LD_{50} (♂)>5000 mg/ kg b.w.
LD_{50} (♀)>5000 mg/ kg b.w.
LD_{50} (combined) >5000 mg/kg bw.
Metabolite Reg. N° 292 932 is not considered harmful by oral uptake in the rat.

Guidelines: Protocol in compliance with test method B.1 of directive 92/69/EEC
GLP status: The study is GLP.

Materials and methods:
5 rats/sex (Wistar, Chbb: Thom(SPF)) received Reg N° 292 932 (purity 99.6; B.n° 00436-69) suspended in 0.5% Tylose (Na Carboxymethylcellulose) by gavage, at a dose level of 2000, 3000 or 5000 mg/kg b.w. (dose volume 20 mL/kg).
No *preliminary experiment*, an initial limit dose of 5000 mg/kg b.w. was selected and extended with the doses of 2000 and 3000 mg/kg b.w. following the death of 1♀.The study is accepted.

B.6.8.4.2 Bacterial gene mutation studies on the metabolites

-Reg. n° 262 451, *S. typhimurium* (TA98, TA100, TA1535, TA1537), and *E. coli* (WP2*uvrA*), plate incorporation[1] or preincubation[2] assay, 0.02 to 5 mg/plate[1] or 0.004 to 2.5 mg/plate[2], ±S9 (Engelhardt, 1995b)

Report: Study of Reg.No. 262 451 (ZHT test substance No.: 94/520) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test); BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep.; 1995/10409.

Findings:
A reduction of background lawn was observed at doses up to 2500 or 5000 µg/pl, and the number of revertants was occasionaly low at 2500 µg/pl the top-dose in some strains. Hence, the highest possible dose was tested in all test strains.
The test article did not increase the number of spontaneous revertants in any test strain in both the plate incorporation and the pre-incubation assay, both in the presence and in the absence of S9.
The positive controls induced the expected number of revertants, with and without metabolic activation.

Conclusion:
Reg. N° 262 451 is not mutagenic in these experimental conditions.

Guidelines: Protocol in compliance with test method B.13/14 of directive 92/69/EEC
GLP status: The study is GLP

Materials and methods:
Overnight grown bacteria from the strains of *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *E. coli* WP2*uvrA* were treated at 37°C during 48h or pre-incubated (20') in the presence of Reg. n° 262 451 (B.n° 00689-145, purity 98.5%; B.n° 00820-251, purity unstated) dissolved in Dimethylsulphoxide (DMSO) at 0; 20; 100; 500; 2500 or 5000 µg/plate (treat and plate *S. typhimurium*) or 0; 4; 20; 100; 500; or 2500 µg/plate (pre-incubation *S. typhimurium;* treat-and-pate and pre-incubation *E. coli*) in the presence and in the absence of S9. The test solutions were checked analytically. *Post-mitochondrial supernatant* was obtained from Aroclor 1254-induced ♂ SD rats, and used for preparation of metabolic activation mixture (10% S9 v:v).
Positive controls were obtained by treating with appropriate reference mutagens as a function of strain and exogeneous activation [–S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine for TA1535 and TA100 (MNNG, 5 µg/pl); 4-nitro-o-phenylenediamine (NPD, 10 µg/pl for TA98); 9-aminoacridine for TA1537 (9-AA, 100 µg/pl); N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 10 µg/pl) and 2-Aminoanthracene (2-AA, 2.5 or 60 µg/pl for all strains, +S9-mix]. Testplates (n=3) were checked for background lawn and presence of precipitations. *Negative controls* were obtained by treating with the vehicle.
Criterium for determining positive response: if a 2-fold increase in incidence of revertants was observed in a dose-responsive way, and if the results were reproducible. The study is accepted.

-Reg. n° 291 685, *S. typhimurium* (TA98, TA100, TA1535, TA1537), and *E. coli* (WP2*uvrA*), plate incorporation[1] or preincubation[2] assay, 0.02 to 5 mg/plate[1,2] or 0.004 to 2.5 mg/plate[2], ±S9 (Engelhardt and Hoffmann, 1995a)

Report: Study of Reg.No. 291 685 (ZHT test substance No.: 94/262) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli / Mammalian-microsome reverse mutation assay (standard plate test and preincubation test); BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep.;1995/10027

Findings:
A reduction of background lawn or reduction of the number of spontaneous revertants was observed at doses up to 500 to 5000 µg/pl. Hence, the highest possible dose was tested in all test strains.
The test article did not increase the number of spontaneous revertants in any test strain in both the plate incorporation and the pre-incubation assay, both in the presence and in the absence of S9.
The positive controls induced the expected number of revertants, with and without metabolic activation.

Conclusion:
Reg. N° 291 685 is not mutagenic in these experimental conditions.

Guidelines: Protocol in compliance with test method B.13/14 of directive 92/69/EEC
GLP status: The study is GLP

Materials and methods:
Overnight grown bacteria from the strains of *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *E. coli* WP2*uvrA*⁻ were treated at 37°C during 48h or pre-incubated (20') in the presence of Reg. n° 291 685 (B.n° 00436-65, purity 97.7%) dissolved in Dimethylsulphoxide (DMSO) at 0; 20; 100; 500; 2500 or 5000 µg/plate (treat and plate *S. typhimurium,* all tests *E. coli*) or 0; 4; 20; 100; 500; or 2500 µg/plate (pre-incubation *S. typhimurium*) in the presence and in the absence of S9. The test solutions were checked analytically. *Post-mitochondrial supernatant* was obtained from Aroclor 1254-induced ♂ SD rats, and used for preparation of metabolic activation mixture (10% S9 v:v).
Positive controls were obtained by treating with appropriate reference mutagens as a function of strain and exogeneous activation [–S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine for TA1535 and TA100 (MNNG, 5 µg/pl); 4-nitro-o-phenylenediamine (NPD, 10 µg/pl for TA98); 9-aminoacridine for TA1537 (9-AA, 100 µg/pl); N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 10 µg/pl) and 2-Aminoanthracene (2-AA, 2.5 or 60 µg/pl for all strains, +S9-mix]. Testplates (n=3) were checked for background lawn and presence of precipitations. *Negative controls* were obtained by treating with the vehicle.
Criterium for determining positive response: if a 2-fold increase in incidence of revertants was observed in a dose-responsive way, and if the results were reproducible. The study is accepted.

-Reg. n° 292 932, *S. typhimurium* (TA98, TA100, TA1535, TA1537), and *E. coli* (WP2*uvrA*), plate incorporation[1] or preincubation[2] assay, 0.02 to 5 mg/plate[1,2] or 0.004 to 2.5 mg/plate[2], ±S9 (Engelhardt, 1995a)

Report: Study of Reg.No. 292 932 (ZHT test substance No.: 94/263) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test); BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep.; 1995/10026.

Findings:
A reduction of background lawn or decreased number of revertants was observed at the top-dose of 5000 µg/pl.
The numbers of revertants was slightly reduced in strain TA100 (–S9)and in *E.coli* at 2500 µg/pl.
The test article did not increase the number of spontaneous revertants in any test strain in both the plate incorporation and the pre-incubation assay, both in the presence and in the absence of S9.
The positive controls induced the expected number of revertants, with and without metabolic activation.

Conclusion:
Reg. N° 262 451 is not mutagenic in these experimental conditions.

Guidelines: Protocol in compliance with test method B.13/14 of directive 92/69/EEC

GLP status: The study is GLP

Materials and methods:
Overnight grown bacteria from the strains of *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *E. coli* WP2*uvrA*⁻ were treated at 37°C during 48h or pre-incubated (20') in the presence of Reg. n° 292 932 (B.n° 00436-69, purity 99.6%) dissolved in Dimethylsulphoxide (DMSO) at 0; 20; 100; 500; 2500 or 5000 µg/plate (treat and plate *S. typhimurium,* all tests *E. coli*) or 0; 4; 20; 100; 500; or 2500 µg/plate (pre-incubation *S. typhimurium*) in the presence and in the absence of S9. The test solutions were checked analytically. *Post-mitochondrial supernatant* was obtained from Aroclor 1254-induced ♂SD rats, and used for preparation of metabolic activation mixture (10% S9 v:v).
Positive controls were obtained by treating with appropriate reference mutagens as a function of strain and exogeneous activation [–S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine for TA1535 and TA100 (MNNG, 5 µg/pl); 4-nitro-o-phenylenediamine (NPD, 10 µg/pl for TA98); 9-aminoacridine for TA1537 (9-AA, 100 µg/pl); N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 10 µg/pl) and 2-Aminoanthracene (2-AA, 2.5 or 60 µg/pl for all strains, +S9-mix]. Testplates (n=3) were checked for background lawn and presence of precipitations. *Negative controls* were obtained by treating with the vehicle.
Criterium for determining positive response: if a 2-fold increase in incidence of revertants was observed in a dose-responsive way, and if the results were reproducible. The study is accepted.

-Reg. n° 339 774, *S. typhimurium* (TA98, TA100, TA1535, TA1537), and *E. coli* (WP2*uvrA*), plate incorporation[1] or preincubation[2] assay, 0.02 to 5 mg/plate[1] or 0.004 to 2.5 mg/plate[2], ±S9 (Engelhardt, 1996)

Report: Study of Reg.No. 339 774, BF490-15 (ZHT test substance No.: 96/28) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test); BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep.; 1996/10353.

Findings:
A reduction of background lawn or decreased number of revertants was observed at the dose of 2500 or 5000 µg/pl, indicating bacteriotoxicity. The test article did not increase the number of spontaneous revertants in any test strain in both the plate incorporation and the pre-incubation assay, both in the presence and in the absence of S9.
The positive controls induced the expected number of revertants, with and without metabolic activation.

Conclusion:
Reg. N° 339 774 is not mutagenic in these experimental conditions.

Guidelines: Protocol in compliance with test method B.13/14 of directive 92/69/EEC

GLP status: The study is GLP

Materials and methods:
Overnight grown bacteria from the strains of *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *E. coli* WP2*uvrA*⁻ were treated at 37°C during 48h or pre-incubated (20') in the presence of Reg. n° 339 774 (B.n° 00956-55, purity 90.2%) dissolved in Dimethylsulphoxide (DMSO) at 0; 20; 100; 500; 2500 or 5000 µg/plate (treat and plate assays) or 0; 4; 20; 100; 500; or 2500 µg/plate (pre-incubation assays) in the presence and in the absence of S9. The test solutions were checked analytically. *Post-mitochondrial supernatant* was obtained from Aroclor 1254-induced ♂SD rats, and used for preparation of metabolic activation mixture (10% S9 v:v).
Positive controls were obtained by treating with appropriate reference mutagens as a function of strain and exogeneous activation [–S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine for TA1535 and TA100 (MNNG, 5 µg/pl); 4-nitro-o-phenylenediamine (NPD, 10 µg/pl for TA98); 9-aminoacridine for TA1537 (9-AA, 100 µg/pl); N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 10 µg/pl) and 2-Aminoanthracene (2-AA, 2.5 or 60 µg/pl for all strains, +S9-mix]. Testplates (n=3) were checked for background lawn and presence of precipitations. *Negative controls* were obtained by treating with the vehicle.
Criterium for determining positive response: if a 2-fold increase in incidence of revertants was observed in a dose-responsive way, and if the results were reproducible. The study is accepted.

B.6.8.4.3 Conclusions on the acute oral toxicity and genotoxicity studies on the metabolites

The plant or soil metabolites Reg. N°. 262 451, 291 685, 292 932 and 339 774 were tested in an acute oral toxicity test in the rat and/or in a bacterial mutagenicity assay (in *S. typhimurium* or *E. coli*). Only metabolite Reg. N°. 262 451 (BF 490-1) was slightly more toxic in the acute oral rat study than the parent compound itself. The LD_{50} of the ♀ was about 1090 mg/kg b.w., and the compound was classified accordingly (Xn; R22). This metabolite was also a major rat metabolite (490 M1, accounting for 4-7% of administered dose in excreta, cfr tables B.6.1.8 and -9). Therefore, the toxicity was covered by existing studies, and the conclusions of the overall assessment remained unchanged. All other metabolites were not harmful by oral uptake.
All investigated metabolites were found not mutagenic in the bacterial assays.
Whereas most of these metabolites were also rat metabolites, Reg. N°. 339 774 was only detected in grapes. However, the latter was structurally very similar to a known rat metabolite (hydroxylated derivative of 490 M1), thus more polar and potentially less toxic.

It was concluded that the present metabolites do not pose a particular problem, and are likely to be covered by the toxicological package of Kresoxim-Methyl itself.

B.6.9 Medical data (Annex IIA 5.9)

B.6.9.1 Report on medical surveillance on manufacturing plant personnel (Annex IIA 5.9.1)

Initial report:
This compound was only developed recently and has not yet been introduced in the market. Kresoxim-methyl was produced in amounts of 3000 kg in the years 1990 to 1995 and roughly 10 persons were involved. No substance related observations were made. Kresoxim-methyl will be produced in Brazil . Each 6 months the personnel will be tested (blood, liver, kidney). One test was already performed. No guideline value was exceeded.

Report at the occasion of the re-submission:

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of Kresoxim-methyl. Thus, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.

The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to Kresoxim-methyl exposure have not been observed.

In 1996, a special cross-sectional health examination study of employed men assigned to Kresoxim-methyl production was conducted (Zober et al., 1997; DocID 1997/10111). Personnel at the Kresoxim-methyl manufacturing site in Guaratinguetá, Brazil, were examined twice at an interval of six months. Employees were subdivided into two main groups: those having no direct contact (N = 47) and those with sporadic or daily contact with the final product (N = 28). Health endpoints of interest were selected based on results of chronic animal studies, which reported indications of liver injury in mice, rats and dogs at high kresoxim-methyl doses. Comparisons of laboratory data were based on ANOVA and regression analyses incorporating a Kresoxim-methyl dose score as the primary explanatory variable and age, smoking history, alcohol intake and body-mass-index (BMI) as concomitant variables.

No findings indicative of Kresoxim-methyl-induced changes in four liver function indicators (gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) were seen at about six months and one year after start-up of the manufacturing process. Individual review of health complaints and conditions also did not reveal a connection between any of these occurrences and assignment to the kresoxim-methyl operations. Under the conditions of exposure, which were not quantitatively characterised, there was no evidence of Kresoxim-methyl-induced health effects.

B.6.9.2 Report on clinical cases and poisoning incidents (Annex IIA 5.9.2)

Some cases of slight irritation of the skin, eyes, mouth or respiratory tract (including rhinitis and cough) have been reported to BASF in persons exposed to Kresoxim-methyl in combination with other products. These reports could not be verified, and it is not clear whether Kresoxim-methyl was the cause for these irritations.

B.6.9.3 Observations on exposure of the general population and epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

The notifier proposes as expected effects of poisoning those deriving from acute and subacute studies in animals:
- Nausea, vomiting, diarrhea (extracted from the study in dogs ?)

- Increase in serum liver enzymes (GGT ?)
- decrease in serum triglycerides, cholesterol and lymphocytes.

It is suggested by the rapporteur to mention the decreases on AP, ALT and AST in spite of the fact that they do not reflect a toxic effect.

In the BASF-internal clinical incident log, some cases of slight irritation of the skin or eyes have been registered, concerning persons exposed to Kresoxim-methyl (in combination with other products). It is not clear whether Kresoxim-methyl was the cause of these irritations.

B.6.9.5 First aid measures - Therapeutic regimes

Symptomatic and supportive. No specific antidote known.

B.6.9.6.1 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Expected effects were derived for acute and subacute studies in animals.

B.6.9.6.2 Expected effects and duration of poisoning as a function of varying time periods - between exposure or ingestion and commencement of treatment.

Expected effects were derived for acute and subacute studies in animals.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL and drinking water limit

Metabolism :
After oral administration in the rat, Kresoxim-methyl showed a rapid but saturable and low **absorption** from the GI tract.
Up to 71% of the parent compound was found in the feces, and biliary excretion accounted for only 14 to 43% of the dose. After parenteral administration, faecal excretion was considerably lower than after ingestion (23-48% versus 71%) and the fraction of the dose excreted via the urine was correspondingly increased. Moreover, when the results obtained at the low and high doses were compared, it became evident that the ratio of fecal versus urinary excretion increased with the dose.
In the original DAR, it was proposed to consider the oral absorption rate 27% at the high dose (500 mg/kg b.w.) and 63% at the low dose (50 mg/kg b.w.), based upon the radioactivity excreted via the urine and the bile. The absorption rate at the low dose is used as a correction factor to establish the AOEL.

In the rat, radioactive material was **distributed** in all tissues and organs throughout the body, and 96 h after dosing the total radioactivity in the organs was less than 2% of the dose administered. The highest radioactivity was associated with the gastro-intestinal tract and the organs of metabolism and elimination, liver and kidney.
After oral administration in the rat, the systemically available proportion of Kresoxim-methyl was rapidly and completely **metabolized.** The ester cleavage, as indicated by the metabolites found in plasma, is the fastest and most important biotransformation step.
Due to the saturable absorption , **excretion** after oral administration mainly occurred, via feces (66-81% of the dose) . After intravenous application, equal amounts were excreted via urine (66%) and feces (48%). There was no evidence of accumulation of radioactive material after repeated dosing.
In vitro dermal absorption using human and rat skin was dose-dependent: after single application of the compound, the rate of penetration of Kresoxim-methyl through rat skin was 3, 1.5 and 2.5 times greater than that observed in human skin at the low, intermediate and high dose levels, respectively.

The major metabolic pathways proposed for the active substance are similar in rats, goats and hens.
An additional minor pathway is , however, observed in hens. Unchanged parent compound was the predominant radioactive constituent in plants. Samples, however, from a rotational crop study did contain , if any, very low amounts of unchanged parent compound, and in these cases a high percentage of conjugated metabolites was identified.

Acute toxicity:
The acute toxicity of Kresoxim-methyl is low. There were no treatment-related morphological alterations in kidneys and liver. After inhalation exposure, male rats presented signs of upper airway irritation (already at a concentration of 2.04 mg/l air) and systemic toxicity (at the concentration of 5.6 mg/l air).Local signs were discharged reddish nose, sounded and irregular respiration, crust formation and bloody nose, reddish and discharged eyes and reddish eyelids with crusts. Signs of systemic toxicity were : high stepping gait, reduced general state , squatting posture and piloerection. The fact that signs of systemic toxicity were seen upon inhalation of a dose of 475 mg/kg over 4 hours, i.e., a dose at least 10 times lower than the highest sign-free, oral dose tested, is in agreement with the partial absorption observed in the toxicokinetic studies after oral administration.
Kresoxim-methyl has no skin or eye irritant properties and is not a sensitiser. It should not be classified for acute toxicity.

Genotoxicity:
Kresoxim-methyl is not genotoxic under the conditions of the assays . It , however, induces some hepatocyte proliferation.

Short- and long-term toxicity:
After subchronic oral exposure at the MTD of kresoxim-methyl, rats showed increases in liver weight, serum albumin and GGT, decreases in the number and the content of fat containing vacuoles in the liver, decreases in serum AP , ALT and AST, without overt signs of hepatotoxicity. At the 28 day MTD dose no alterations could be observed at the electron microscopic level in the liver peroxisomes or mitochondria. Although no clearcut

changes occurred in food intake, which would have explained a decrease in serum AP, additional information suggests that this might be due to a slight interference with fat absorption. Decreases in serum ALT and AST remain more difficult to explain, but being accompanied by an increase in GGT, suggest some metabolic stress. An increase in TSH levels, without changes in T3 or T4 and without enzyme induction, an isolated finding in the 28 day rat test, is interpreted as a chance finding.

Negative results were obtained in a short term interaction study performed to assess whether inhibition of esterases that detoxify Kresoxim-methyl by cholinesterase inhibitors may potentiate the toxicity of Kresoxim-methyl.

Mice responded in a similar way, although the changes were less pronounced.

Dogs exhibit altered food intake, and reacted with vomiting and diarrhea, with impact on body weight and transient decreases in serum albumin and protein concentrations but without signs of organ toxicity.

Kresoxim-methyl has an oncogenic potential at the MTD (8000ppm) in rats and a significant increase in malignant liver tumours was reported . The primary epithelial neoplasms of the liver were cholangiomas, carcinomas, and cholangiocarcinomas. In both sexes, liver tumours occurred exclusively in old animals that survived until the scheduled termination of the study.
The non-neoplastic liver lesions-eosinophilic foci of hepatocellular alterations and hypertrophy of hepatocytes-further indicates metabolic stress on the liver parenchyma. The incidence and degree of severity of minimal to severe bile duct proliferation was increased in female of the high dose group (16000 ppm), while the incidence of fatty change was decreased. In male rats, significant and dose-dependent increase in GGT was found throughout the study at the MTD.

At doses of 200 and 800 ppm, no oncogenic or systemic toxic findings were noted.

At the occasion of the resubmission, the carcinogenicity study was replicated in another strain of Wistar-rats (Crl Glx Brl Han:WI) at the control and top-dose of 16000 ppm, under the assumption that the latter strain would be less sensitive to the induction of liver tumours than the initially used one (Chbb:Thom).
Liver carcinoma occurred at higher incidence in both the ♂ and the ♀ and the incidence in the new study was significantly lower in the ♀, as compared to the former study.
Therefore, the new results did not alter the former conclusion, being that Kresoxim-methyl was to be considered carcinogenic towards the rat (Carc. Cat. 3-Xn; R40).

As described above, Kresoxim-methyl has no genotoxic properties and does not initiate the formation of liver foci as seen with the cancer initiator N-nitrosomorpholine. At carcinogenic doses it produced hepatic cell proliferation together with mild hepatic toxicity, both being reversible. On the basis of all data presented it can be concluded that Kresoxim-methyl is a non-genotoxic carcinogen in the rat, acting as a promotor for which a threshold dose exists.

In C57BL-strain mice, the test article was not oncogenic under the conditions of this assay. Papillary necroses of the kidneys and increased number of females with amyloidosis of the liver associated with a higher degree of severity in females exposed to 8000 ppm were considered as treatment related .

Reproductive toxicity and carcinogenicity :
Kresoxim-methyl had no adverse effects on reproductive parameters of rats , but produced some dose - dependent developmental toxicity in the pups, impaired body weight/body weight gain and some indications for delays in the morphological development at doses which produced clear parental toxicity. The overall and developmental NOAEL were 100 and 1500 mg/kg bw/d, respectively.

No substance related adverse effects were observed in pregnant rats and Himalayan rabbits and there were no indications of embryo-/fetotoxicity and especially no substance-induced signs of teratogenicity .

Neurotoxicity

At the occasion of the resubmission of the dossier, acute and subchronic (90d) neurotoxicity studies were performed. In the acute neurotoxicity study, where Kresoxim-Methyl was administered up to and including the limit dose of 2000 mg/g b.w., no neurotoxic adverse effects were observed. When the compound was administered in the food during 90d up to and including 16000 ppm (1180 mg/kg b.w./d), slight systemic toxicity (decrease of food consumption and of body weight) was observed at the top-dose, but neurotoxic effects were not identified. It was concluded that the compound was devoid of neurotoxic potential.

Metabolites
The metabolites Reg. N°. 262 451, 291 685, 292 932 and 339 774 were tested in an acute oral toxicity test in the rat of in a bacterial mutagenicity assay (in *S. typhimurium* or *E. coli*). Only metabolite Reg. N°. 262 451 (BF 490-1) was slightly more toxic in the acute oral rat study than the parent compound itself. The LD_{50} of the ♀ was about 1090 mg/kg b.w., and the compound was classified accordingly (Xn; R22). As this metabolite was also a major rat metabolite, the toxicity was covered by existing studies, and the conclusions of the overall assessement remained unchanged. All other metabolites were not harmful by oral uptake.
All investigated metabolites were found not mutagenic in the bacterial assays.
Most of these metabolites were also rat metabolites, except Reg. N°. 339 774, which was only a grape metabolite. However, the latter was structurally very similar to a known rat metabolite.
It was concluded that the present metabolites do not pose a particular problem, and are likely to be covered by the toxicological package of Kresoxim-Methyl itself.

B.6.10.1 Establishment of an Acceptable Daily Intake (ADI)

Consumer exposure to Kresoxim-methyl will be mainly orally with food intake.

It appears from the animal experiment that a chronic exposure to 36 mg/kg bw/day (lowest NOAEL, taken from the 2 year rat study) of Kresoxim-methyl will not result in any toxic effect. At a higher dose hepatotoxic effects were seen. A lower NOAEL in the short term cell proliferation studies is not taken into account since the LOAEL in that study is higher than the NOAEL in the 2 year rat study.

The critical toxic effect in the rat is an hepatic toxicity resulting in an increased occurrence of hepatocarcinomas. Kresoxim-methyl is not genotoxic, the carcinogenic effect only occurs in old rats and seems to be mediated via induction of cell proliferation. It means that below an active dose, the potential to induce tumours is negligible. Therefore, but because of the tumour effect, an irreversible second end-point, an uncertainty factor of 100 is proposed for the extrapolation to man:

ADI = 0.4 mg/kg bw/day

This ADI is about 900× lower than the LOAEL producing liver tumours in the rat (370 mg/kg b.w./d), which seems sufficient for a non-genotoxic carcinogenic substance.
In the original DAR, a safety factor of 250× was proposed, but decreased to 100× during the subsequent ECCO-meeting.

B.6.10.2 Establishment of an Acceptable Operator Exposure Level (AOEL)

The AOEL for man is calculated on the basis of an internal NOAEL from a sub-chronic animal experiment, taking into account the apparent degree of absorption, and applying an uncertainty factor, chosen in function of the critical effect observed in the animal experiments.

It appears from the animal experiment that a subchronic exposure to 140 mg/kg bw/day (lowest NOAEL, taken from the 1 year dog) of Kresoxim-methyl will not result in any toxic effect. This value is supported by the 90d study in rats (146 mg/kg b.w./d). At higher dose hepatotoxic effects are seen.
It further appears from the ADME studies that only part of the Kresoxim-methyl orally ingested is absorbed, a figure of about 63% at the dose of 146 mg/kg bw/day is proposed. The internal dose at that oral intake, therefore, equals 92 mg/kg bw/day.

The critical toxic effect in the rat is an hepatic toxicity resulting, in the long term study, in an increased occurrence of hepatocarcinomas. Kresoxim-methyl is not genotoxic, the carcinogenic effect only occurs in old rats and seems to be mediated via induction of cell proliferation. It means that below an active dose, the potential to induce tumours is negligible. Therefore, but because of the tumour effect, an irreversible second end-point, an uncertainty factor of 100 is proposed for extrapolation to man.

AOEL = 0.9 mg/kg bw/day

The AOEL is about 400× lower than the LOAEL producing liver tumours in the rat, which is sufficient for a non genotoxic carcinogenic substance.

B.6.10.3 Establishment of an Acute Reference Dose (ARfD)

Due to the low acute oral toxicity of the active ingredient, and the absence of adverse effects in prenatal toxicity studies or in the acute neurotoxicity study, an ARfD is not considered to be required. An acute Reference Dose (ARfD) has not been allocated for Kresoxim-methyl in the course of the previous EU authorization process and there has been no new data that would require allocation of an ARfD.

B.6.10.4 Establishment of the (theoretical) drinking water limit.

The maximum admitted concentration, as established in the guideline 80/77/EC, is 0.0001 mg/L.

Studies on the representative formulations

Two representative formulations are presented by the notifier: Candit (50% WG) and Allegro (12.5% SC)

Withdrawn formulation: MENTOR
MENTOR is the proposed commercial name for BAS492 01F (suspension-emulsion containing 150 g/l kresoxim-methyl and 300g/l fenpropimorph) BASF
Added March 2010: the formulation MENTOR is not supported any more

CANDIT

CANDIT is the commercial name for BAS 490 02 F (WG containing 500 g/kg kresoxim-methyl) BASF.

B.6.11.1a Acute oral toxicity (Annex IIIA 7.1.1.1)

- Report: study on the acute oral toxicity of BAS 490 02 F in rats (Kirsch et al.,1994b, Dossier BASF)
Guidelines :
Experimental protocol in compliance with the test B.1 of Directive 92/69/EEC or Diective 84/449/EEC or OECD 401 (1981-1987) :
GLP status : yes
Material and methods:
5 Wistar (CHBB:THOM,SPF) rats/sex received a single dose of BAS 490 02 F (lot n°.92-5) as a dispersion in aqua bidest.at a dose level of 5000mg/kg bw.
Findings :
signs and symptoms : impaired general state, compulsary gnawing, diarrhea, salivation, in 1 female .Another 3 females and 1 male rat exhibited diarrhea.
mortality : no dead.
bw determination: no abnormality.
pathology : no pathologic findings.
Conclusion :
The animals appeared normal 3 hours after application.
LD50 M +F> 5000 mg/kg bw

B.6.11.2a Acute dermal toxicity (Annex IIIA.7.1.2)

- Report: study on the acute dermal toxicity of BAS 490 02 F in rats (Kirsch, et al.,1994c, Dossier BASF)
Guidelines:
Protocol in compliance with method B.3 of Directive 92/69/EEC.
The study is GLP
Material and methods :
5 Wistar rats (CHbb:THOM(SPF))/sex were exposed to BAS 490 02 F (lot n°.92-5)at a dose level of 2000mg/kg bw, (4 ml/kg) by dermal semi-occlusive application for 24 h.
Findings :
Mortality: no dead
Clinical observations: no signs of systemic toxicity and no local effects.
bw determination: no abnormality.
Necropsy: no abnormalities noted.
Conclusion:
Under the conditions of this study , the LD50> 2000mg/kg bw.

B.6.11.3a Acute inhalation toxicity to rats (Annex IIIA.7.1.3)

- Report: study on the acute inhalation toxicity LC50 of BAS 490 02 F as a dust aerosol in rats -single 4-hour exposure (Gamer and Kirsch, 1994, Dossier BASF)

Guidelines:
Protocol in compliance with the method B.2 of the Directive 92/69/EEC.
GLP status : yes
Material and methods:
5 Wistar rats (CHbb:THOM(SPF))/sex were exposed for 4 h by head-nose inhalation exposure to BAS 490 02 F (B.n°.92-5) at 5.7 mg/l. The particle size distribution yielded mass median aerodynamic diameters of 2.8 µm which is in a well respirable range.(respirability : 91%)
Findings :
Mortality : no dead
Clinical signs : accelerated respiration during exposure and fur contamination on day 0.
Body weight : unchanged
Necropsy : no abnormalities
Conclusion:
No lethality nor abnormalities were detected in the animals of the test group after exposure.
LC50 > 5.7 mg/l

B.6.11.4a Skin irritation (AnnexIIIA 7.1.4.)

- Report: study on the acute dermal irritation/corrosion of BAS 490 02 F in the rabbit (Rossbacher and Kirsch, 1994c, Dossier BASF)
Guidelines:
Protocol in compliance with the test B.4 of Directive 92/69/EEC.
GLP status :yes
Material and methods :
6 White Vienna rabbits were exposed to 0.5 g of BAS 490 02 F(lot n°.92-5) applied to the intact skin and covered with a semi-occlusive dressing during 4 h.
Findings:
Evaluation of the data, according to the EU methodology, gave the following results:
<Score erythema>$_{24+48+72\ h}$ = 0.1
<Score oedema >$_{24+48+72\ h}$ = 0
The skin reactions were reversible within 48 h.
Conclusion :
BAS 490 02 F has no irritant properties and is therefore not classified.

B.6.11.5a Eye irritation (Annex IIIA 7.1.5)

- Report: study on the acute eye irritation of BAS 490 02 F in the rabbits (Rossbacher and Kirsch, 1994d, Dossier BASF)
Guidelines:
Protocol fully in compliance with the method B.5 of Directives 92/69/EEC .
GLP status: yes
Material and methods :
37 mg of BAS 490 02 F (lot n°.92-5) was applied into the conjunctival sac of the right eyelid of 6 New white Vienna rabbits.
Findings:
<Score cornea opacity.>$_{24+48+72\ h}$ = 0.0
<Score iris> $_{24+48+72\ h}$ =0.0

<Score erythema> 24+48+72 h =0.2
<Score chemosis> 24+48+72 h =0.0

The findings were reversible within 48 h in all animals.

Conclusion :

Under the test conditions chosen BAS 490 02 F does not give indication of an irritant property to the eye.

B.6.11.6a Skin sensitization (Annex IIIA 7.1.6)

- Report: on the Buehler test for the sensitizing potential of BAS 490 02 F in guinea-pigs. (Rossbacher and Kirsch, 1994e, Dossier BASF)

Guidelines:

Protocol in compliance with the method B.6 of the Directive 92/69/EEC .

GLP status : yes (no attest of competent authority).

Material and methods :

Pretest: 4 Female Guinea-pigs (Pirbright strain) /dose were exposed to 0.5 ml of BAS 490 02 F(lot n°.92-5) during 6 h, 2 times.

Main test:20 Female Guinea-pigs (Pirbright strain) received 3 inductions with 0.5 ml (60%)of BAS 490 02 F(lot n°.92-5) during 6 h on day 0, 7 and 14. A challenge was carried out 14 days after the third induction with 0.5 ml (60%) of the test substance formulation. A second group with 10 animals served as a control group.

Findings :

The induction with the 60% test substance preparation did not cause any skin reactions in the animals of the test group. The controls were not treated because aqua bidest. was used as vehicle. The challenge with the 60% test substance preparation did not cause any skin reactions neither in the control nor in the test animals.

sensitization rate : 0%

Conclusion :

CANDIT does not have a sensitizing effect on the skin in the Buehler test.

B.6.11.7a Additional studies for combinations of plant protection products (Studies as at points 7.1.1 to 7.1.6) (Annex IIIA 7.1.7)

No data submitted

B.6.11.8a Summary of the toxicity of the formulation CANDIT

Table B.6.14a -1 : Summary of toxicological data of CANDIT

test	species	results	classification	references
acute oral	rat	> 5000 mg/kg bw	-	Kirsch et al.,1994b
acute dermal	rat	> 2000 mg/kg bw	-	Kirsch, et al.,1994c
acute inhalation	rat	> 5.7 mg/l	-	Gamer and Kirsch, 1994
skin irritation	rabbit	not irritant	-	Rossbacher and Kirsch, 1994c
eye irritation	rabbit	not irritant	-	Rossbacher and Kirsch, 1994d
skin sensitization Buehler test	guinea-pig	not sensitiser	-	Rossbacher and Kirsch, 1994e

According to Dir. 88/379/EEC, CANDIT, containing Kresoxim-methyl which is classified in cat. 3 for carcinogenicity, needs also to be classified in Cat. 3 and labelling will be adapted consequently.

B.6.12 a Dermal absorption

B.6.12.1a Dermal absorption, *in vivo* in the rat (Annex IIIA 7.3)

A skin penetration study on human and rat skin *in vitro* with Kresoxim-methyl, formulated as a 50% WG was performed. No data on dermal absorption study *in vivo* exists.

B.6.12.2a Comparative dermal absorption, *in vitro* using rat and human skin (Annex IIIA 7.3)

Original DAR:
For the purpose of operator exposure calculations, a default 10% rate of dermal absorption was considered, as no study existed at that time.
At the occasion of the re-submission of the dossier, a new *in-vitro* dermal absorption study with BAS 490 02F (50% WG Kresoxim-Methyl) was conducted on human skin. The results are evaluated as follows:

-percutaneous absorption, split-thickness skin *in-vitro*, Kresoxim-Methyl (BAS 490 02 F, formulated as a 50% WG product), human skin, 2500 µg/cm² -concentrate and 8 or 0.6 µg/cm² -spray dilutions (Gamer and Landsiedel, 2008)

Findings:
The *in-vitro* skin absorption of Kresoxim-Methyl, formulated as a 250 g/L formulation (maximal attainable concentration) was assessed in human split-thickness skin, at three representative concentrations (neat formulation and two field dilutions) for 24h.
From the cumulative levels (see table B.6.12.2-1), the steepest increase was observed 0-2h (mid-dose) and 2-4h (low-dose, concentrate) following the start of the experiment. The absorption attained a plateau at about 10h for the concentrate, while a shallow increase was still observed during the 10h-24h sampling time for the mid- and the low dose.
The permeability coefficients (Kp) calculated for the mid- and low-dose were comparable (factor 2), but considerably higher than for the concentrate. The lag-periods were 0.7-1.2h.

Table B.6.12.2-1: *In-vitro* mean cumulative amounts of Kresoxim-Methyl (50% WG) through human epidermis

Dose (µg/cm²)		**concentrate**	**mid**	**low**
target		**2500**	**8**	**0.6**
nominal		**2652**	**8.07**	**0.628**
time (h)	**in % of applied radioactivity**			
0.5		0.0059	0.038	0.012
1		0.0115	0.202	0.057
2		0.0334	1.231	0.391
4		0.0984	2.634	1.423
6		0.1535	3.200	2.351
10		0.1994	4.112	3.072
24		0.2035	5.026	3.873
time (h)	**in µg/cm²**			
0.5		0.158	0.0031	0.0001
1		0.306	0.0163	0.0004
2		0.887	0.0992	0.0025
4		2.613	0.2121	0.0089
6		4.075	0.2575	0.0148
10		5.287	0.3310	0.0193
24		5.392	0.4047	0.0243
	flux (µg/cm²/h)	0.903	0.083	0.0033
	Kp (cm/h) × 10^{-5}	0.341	10.0	5.16
	lag time (h)	0.7	0.8	1.2

Recovery values (see table B.6.12.2-2) at termination were acceptable, reaching about 105-109% of dose. It was observed that a non-negligeable amount of radioactivity was still present in the skin tissue. According to the guideline, and since skin depot may have constituted a reservoir from which further systemic exposition could occur, the amounts should be taken into account for the determination of the skin absorpion.
Hence, absorption values of both acceptor fluid and of tissue samples at 24h were summed up. For human skin, total values amounted to 0.262%, 9.13% and 6.75% of the dose, for respectively the neat formulation, the mid- and low field-dilution.

Table B.6.12.2-2: Recovery (expressed in % of applied dose) of Kresoxim-Methyl (50% WG) at 24h

	Dose (µg/cm²)	**concentrate**	**mid**	**low**
	target	**2500**	**8**	**0.6**
	nominal	**2652**	**8.07**	**0.628**
Non-absorbed	**tape strips (N=6)**	0.037	4.09	2.84
	skin rinse 6h	104.5	87.1	91.5
	24h	0.333	8.48	6.89
	sum non-absorbed	104.9	99.7	101.3
Absorbed	**skin depot**	0.047	2.59	1.45
	intermittent samples+flush out	0.075	1.73	1.50
	final receptor fluid	0.121	3.55	2.57
	receptor chamber wash	0.019	1.26	1.23
	sum absorbed*	**0.262**	**9.13**	**6.75**
	total recovery	105.1	108.8	108.0

*: taking into account the skin depot (=skin – 6 tape strips)

Conclusion:
The absorption value of Kresoxim-Methyl, formulated as a 50% WG product in human skin, as assessed in an *in-vitro* assay, was considered 0.3 % for the neat formulation, and 9% for the (worst-case) in-use field dilution.
It is proposed to use these values to assess the operator exposure to the representative Kresoxim-Methyl formulation.

Guidelines: No EU guideline exists, but the study is in line with the OECD Draft Guideline 428
GLP-status: the study is GLP

Materials and methods:
Five skin membranes/dose from 4 human donors (2/dose) were exposed to Kresoxim-Methyl (97.8%; B.n° COD-000225), formulated as a 48.2% WG, and mixed with radiolabelled compound ([ϕ-U-^{14}C]-Kresoxim-Methyl, radiochemical purity 99.2%; B.n° 613-2101, specific activity 13.1 MBq/mg). The blank formulation was mentioned as BAS 490 AA F, B.n° Ans.-nr. 992003. Tap water was used to prepare the spray dilution.
The skin membranes were obtained by *split-thickness skin* dermatome cutting at about 350-450 µm.
Achieved doses were as tabulated below (250, 0.8 and 0.06 mg/mL applied, since the applied volume was 10 µL/cm²).
The neat formulation is a 25% suspension (maximal technically achievable dilution of a 50% WG), thus 250 g/L. The field dilutions are as follows:
(i) mid-concentration: 0.3 kg product (0.15 kg a.s.) / 150 L, thus 1 g a.s./L., and
(ii) low concentration: 0.2 kg product (0.1 kg a.s.) a.s./ 1800 L, thus 0.056 g a.s./L.

The concentrations, expressed in µg/cm² (means ± s.d.) are nominal or actual values:

Dose group	**nominal**	**actual**
neat formulation	2500	2652 ± 46.3
mid concentration	8	8.07 ± 0.21
low concentration	0.6	0.628 ± 0.011

The product was applied on skin membranes which were fixed in diffusion chamber. The duration of application was 6h, followed by removal of the product from the membrane and a further 18h incubation. Each chamber consisted of 2 compartments, with the upper compartment (side of the stratum corneum, exposed to air) for the topical application of the

test article, and the lower compartment with the receptor fluid (ethanol:water 50% v:v concentrate, 30% v.v. mid-dose and 0% low-dose, maintained at 32±1°C). The solubility, stability and homogeneity of the test substance in the preparations was determined and found suitable.
Only membranes, tested for integrity by electrical resistance determination (in the range of 1 kΩ) were used for the study (permeability coefficients were thus not determined before the test).
Samples (about 600 µL) were taken at intervals of 0.5, 1, 2, 4, 10 and 24h, whereby receptor fluid was refilled with the same amount. Mass balance was determined at termination, by collecting remaining test article, skin membranes, receptor fluid, and dish-wash, and assessing radioactivity by liquid scintillation count. In addition, the washed skin was tape-stripped in order to remove the stratum corneum (6 tape-strips). The amount of radioactivity in both the stratum corneum and the remaining skin were analysed separately, and only the amount in the latter was considered part of the absorbed dose.
Radioactivity was expressed as % of applied dose (relative skin absorption). Skin penetration rate was estimated by calculating the slope of the penetration-time curve, and was expressed as the flux constant (in µg/cm²/h). The permeability coefficient Kp (in cm/h) was calculated as the flux constant / applied concentration (µg/cm³). The lag phase (h) was calculated from the intersection of the "steady-state" penetration-time curve with the X-axis.
No reference control (such as testosterone) was used as a positive control.
The study is accepted.

B.6.13a Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

No data, not necessary.

B.6.14a Exposure data (Annex IIIA 7.2)

BAS 490 02 F ("Candit") is formulated as a wettable granule (WG) formulation containing 500 g/kg Kresoxim-methyl. The preparation will be used as fungicide in grapes and pome fruits and will be applied up to 4 times per season. BAS 490 02 F will be used at growth stages 19-81 of the crops. The maximum application rates are 0.3 kg/ha for grapes and 0.25 kg/ha for pome fruits, corresponding to 0.15 kg and 0.125 kg a.s./ha, respectively. Recommended spray volumes ranged from 150 to 1800 L/ha.

Applications of BAS 490 02 F will be performed using tractor-mounted air-blast sprayers for field applications in grapes and pome fruits. In addition, for applications in grapes the use of hand-held equipment (lance or spray gun that is connected via a hose to a large mobile tank, or a standard knapsack sprayer) can be considered. Therefore, the assessment of operator exposure and risk evaluation is made considering these application techniques. Usage information pertinent to operator exposure is summarised in Table B.6.15-1.

B.6.14.1a Estimation of operator exposure (Annex IIIA 7.2.1.1)

The dermal absorption values for respectively the neat 50% WG formulation BAS 490 02F ("Candit") and the spray mix were considered 0.3% and 9%. The obtained predicted exposure was compared with the appropriate AOEL (0.9 mg/kg b.w./d) and expressed as % of AOEL.

B.6.14.1.1a Estimation of operator exposure according to UK-model

The application parameters used for the calculation in the UK-model are outlined in table B.6.14.1.1a-1.

Table B.6.14.1.1a-1:
Application parameters of Kresoxim-Methyl (formulation BAS 490 02 F, "Candit") for the UK-exposure model

Formulation		**BAS 490 02F**
Intended uses		pome fruits (apple, pear), grapes
work rate	(ha/d)	15
application rate	(kg product/ha)	0.3
application volume	(L spray mix/ha)	150
application equipment		• orchard (tractor drawn assisted orchard sprayer 500L/ha) • low level hydrolic knapsack sprayer model – 15L spray tank

The estimated operator exposure to Kresoxim-Methyl in the UK POEM model is summarised in table B.6.14.1.1a-2 (see also details in Appendix C).

(i) For the tractor-mounted application, the operator exposure was estimated to amount 0.188, 0.187 or 0.133 mg/kg b.w./d, corresponding with about 20.9%, 20.8% or 14.8% of the proposed AOEL, respectively in the absence of PPE, in the presence of PPE during mixing/loading, or in the presence of PPE in mixing/loading and application.
(ii) For the hand-held application, the operator exposure was estimated to amount 0.158, 0.155 or 0.076 mg/kg b.w./d, corresponding with about 18%, 17% or 8% of the proposed AOEL, respectively in the absence of PPE, in the presence of PPE during mixing/loading, or in the presence of PPE in mixing/loading and application.

Table B.6.14.1.1a-2: Predicted exposure to Kresoxim-Methyl in UK-POEM

	PPE absent	**PPE present**	
		M/L	**M/L + application**
(i) orchard (tractor drawn assisted orchard sprayer 500L/ha)			
estimated exposure (mg/kg b.w./d)	0.1881	0.1868	0.1328
% of AOEL	**20.9**	**20.8**	**14.8**
(ii) low level hydrolic knapsack sprayer model – 15L spray tank			
estimated exposure (mg/kg b.w./d)	0.1575	0.1550	0.0763
% of AOEL	**18**	**17**	**8**

M/L: mixing and loading task

It was concluded that the estimated exposure of an operator in a tractor-mounted orchard spray model or hand-held application was acceptable in all scenarios of application in the UK POEM model, including those where no PPE would be worn.

B.6.14.1.2a Estimation of operator exposure according to the German model

Table B.6.14.1.2a-1:
Application parameters of Kresoxim-Methyl (formulation BAS 490 02 F, "Candit") in the German model

Application method	**Tractor high crops**	**Hand high crops**
Formulation type	WG	WG
a.s. concentration	500	500
Dermal absorption (%) from product	0.3%	0.3%
spray	9%	9%
Application rate (kg/ha product)	0.3	0.3
Work rate/day (ha)	20	1
Amount a.s. (kg) handled /day	3	0.15

In summary, in the tractor high-crop model, the operator exposure was estimated to amount 0.046 or 0.0076 mg/kg b.w./d, corresponding with about 5.1% or 0.9% of the proposed AOEL, in the absence of PPE, or in the presence of PPE in mixing/loading and application, respectively
In the hand high-crop model, the operator exposure was estimated to amount 0.0086 or 0.0019 mg/kg b.w./d, corresponding with about 0.96% or 0.21% of the proposed AOEL, in the absence of PPE, or in the presence of PPE in mixing/loading and application, respectively (see also details in Appendix C).

Table B.6.14.1.2a-2: Predicted exposure in to Kresoxim-Methyl in the German model

	Tractor		**Hand-held**	
	PPE absent	**PPE* present M/L + application**	**PPE absent**	**PPE* present M/L + application**
estimated exposure (mg/kg b.w./d)	0.04573	0.007624	0.008612	0.001874
% of AOEL	**5.08**	**0.85**	**0.96**	**0.21**

* PPE including gloves during M/L and application, coverall and boots during application

It was concluded that the estimated exposure of an operator in a tractor-mounted orchard or hand-held spray model, was acceptable in all scenarios of application in the German model, including those where no PPE would be worn.

General conclusion:
The handling of a 0.8 g/L formulation of Kresoxim-Methyl during mixing/loading and high-crop application of grapes or pome fruit is not expected to pose an undue risk to the operators, even when no PPE would be worn. However, as the a.s. is a Cat. 3 carcinogen, the wearing of PPE should be recommended.

B.6.14.2a Measurement of operator exposure (Annex IIIA 7.2.1.2)

No field studies have been performed with the present Kresoxim-Methyl formulation, as no exceedence of the proposed AOEL was anticipated, according to the existing operator exposure models.

B.6.14.3a Estimation of bystander exposure (Annex IIIA 7.2.2)

B.6.14.3.1a Bystander estimation according to the Lloyd and Bell model

In this calculation, the same parameters (application rate, spray volume, absorption rate, body weight) were considered as for the UK POEM operator exposure. Using these assumptions following exposure may be predicted:

Table B.6.14.3.1: Predicted exposure of the bystander to formulation BAS 490 02 F, "Candit" in orchard crop spraying, according to the model of Lloyd and Bell

ACTIVE SUBSTANCE	**Kresoxim-Methyl**	
PRODUCT	**Candit**	
PARAMETERS		
	Dermal exposure	***Inhalation exposure***
Volume of spray solution dermally intercepted (mL)	3.7	-
Volume of spray solution intercepted by inhalation (mL/m^3)		0.002
Spray volume (L/ha)	150	150
Breathing rate (m^3/hour)	-	3.6
Number of hours worked/day	-	0.08
Application rate (g/ha)	150	150
Percent absorbed (%)	9	100
CALCULATIONS		
	Dermal exposure	***Inhalation exposure***
Dermal intercepted	0.002467%	
Inhalation intercepted		0.000000384%
Amount active intercepted (mg)	3.7	0.0001659
Absorbed dose (mg)	0.333	0.0001659
Bystander weight (kg)	60	60
Absorbed dose (mg a.s./kg bw/d)	0.00555	0.0000027648
Total systemic (mg a.s./kg bw/d)*	0.005553	
AOEL (mg/kg bw/d)	0.9	
Exposure as a ratio of the AOEL:	**0.62%**	

*: sum of dermal and inhalatory exposure, values expressed as % of AOEL

Using this model, a total (dermal+inhalatory) exposure of about 5.6 µg a.s./kg b.w./d would be expected, corresponding to about 0.62% of the AOEL.

Reference: Lloyd GA, Bell GJ, Samuels SW, Cross JV and Berrie AM, Orchard sprayers: comparative operator exposure and spray drift study, MAFF 1987 [Orchard]

B.6.14.3.2a Bystander estimation according to the Ganzelmeier model

For the calculation of the bystander exposure *(Ganzelmeier, 1995)*, worst-case application rates were supposed. The maximal value active substance per ha was considered, i.e. 0.15 kg a.s./ha.

(i) Potential dermal exposure

The bystander theoretical dermal exposure D was estimated according to the following relationship:

D = 100% deposition × drift deposition × exposed area,

where:
- the *100% deposition* equals the application rate:
= 0.15 kg a.s./ha or 15 mg a.s./m²

- *drift deposition*, for a 7.5 m distance in a high crop was estimated 2.6%, and,

- *exposed area* is normally estimated 0.4225 m²/person/day (exposed area includes: head, back and front of neck, forearms, $^1/_2$ upperarms and hands; working day about 6h).

However, it could be remarked that a bystander clothing would not confer a 100% protection to the spray drift. Therefore, it was considered that the remaining body area (2 – 0.4225) m²= 1.5775 m², protected by normal clothing would be exposed to 20% (default exposure mitigation factor) of the drift in a worst-case assumption.

Based upon these assumptions, the bystander dermal external exposure was:
D = (1 ×15 mg/m² × 0.026 × 0.4225 m²/person/day) + (0.20 × 15 mg/m² × 0.026 × 1.5775 m²/person/day)
= 0.164775 + 0.123045
= 0.28782 mg Kresoxim-Methyl /person/day.

With a bystander bodyweight was of 60 kg, the dermal external exposure would amount to 0.004797 mg/kg b.w./d.
Taking into account a dermal absorption of 9%, the internal exposure would calculate 0.000432 mg/kg b.w./d.

(ii) Potential inhalation exposure
The bystander theoretical inhalation exposure was estimated according to the following relationship:

$I = I_A \times WR \times AR$,
where:
- I_A corresponds to the specific exposure during application, estimated 0.018 mg/kg a.s./person in high crops,
- *WR* is the working rate, 8 ha/d for high crops
- *AR* is the application rate, 0.15 kg a.s./ha for Kresoxim-Methyl.

Thus, I = 0.018 mg/kg a.s./person × 8 ha/d × 0.15 kg a.s./ha,
= 0.0216 mg / person / d

According to these values, the bystander maximal theoretical inhalation exposure was 0.00036 mg /kg b.w./d or 0.36 µg / kg b.w./d (assuming a default body weight of 60 kg for the bystander).

If the value was adapted to a 5 minute exposure for a bystander (instead of 6h for the operator exposure, thus dividing by 360/5=72), the expected absorbed dose by inhalation would be 0.000005 mg/kg bw, which is negligible when compared with the potential dermal exposure.

Considering the exposure about 0.000437 mg/kg b.w./d, about 0.049% of the AOEL of Kresoxim-Methyl would be used up.
In conclusion, the risk for the bystander is expected to be very low.

Reference:
Ganzelmeier et al. Studies on the drift of plant protection products. Results of a test program carried out throughout the Federal Republic of Germany. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Heft 305, 1995

B.6.14.4a Estimation of the worker exposure (Annex IIIA 7.2.2)

Kresoxim-Methyl formulated as "Candit" (BAS 490 02F), is used as a fungicide, in pome fruits and grapes, and worker exposure during girdling/tying, pruning and picking activities should be assessed. Therefore, a calculation was performed using the German approximation (Hoernicke et al, 1998), using the parameters as agreed for the EUROPOEM database. Further, a work duration lasting 8 h/d, and a penetration factor of 100% (assuming that PPE would not be worn in this phase) was proposed in a worst-case assumption.

The worker exposure (per kg b.w.) may be estimated by means of the following equation:

D = DFR × TF × WR × AR × P × A ÷ b.w.,

where:
-DFR is the dislodgeable foliar residue,

-TF is the transfer factor,
-WR is the working rate,
-AR is the application rate,
-P is the penetration factor (through protective clothing),
-A is a dermal absorption of 100% (field dilution), and
- b.w. is the worker's body weight.

Assuming a DFR	=0.003 mg a.s./cm² (Europoem II, 2002),
a transfer factor TF (high-crop estimation)	=10000 cm²/person/h (Europoem II, 2002),
a working rate	=8h/d,
a default penetration factor (PPE not used)	=100%,
an application rate AR	= 0.15 kg a.s./ha
a skin absorption	=9%, and
a body weight	=60 kg.

D calculates as follows:

D = 0.003 mg a.s./cm² × 10000 cm²/person/h × 8h/d × 0.15 kg a.s./ha × 1 × 0.09 × 1/60 kg
= 0.054 mg a.s./kg b.w./d

The potential dermal exposure of about 0.054 mg/kg b.w./d, accounts for about 6% of the AOEL.

The use of PPE would consequently further reduce the systemic exposure.

Thus, it was considered that the exposure to Kresoxim-Methyl under the current GAP, of persons entering freshly pulverised crops (for a duration of 8h/d), would be acceptable.

References:

-Hoernicke, Nolting, Wastphal, IVA-Fachausshuss Anwenderschutz; Hinweise in der Gebrauchsanleitung zum Schutz von Personen bei Nachfolgearbeiten in mit Pflanzenschutzmitteln behandelten Kulturen (worker re-entry). Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 50, Berlin, 1998.
-The development, maintenance and dissemination of generic European databases and predictive exposure models to plant protection products. A Concerted Action under area 4 of FAIR, the Fourth Framework (Agriculture and Fisheries including Agro-Industry, Food Technology, Forestry, Aquaculture and Rural Development) specificCommunity Research and Technological Development Programme. FAIR3 CT96-1406. Finalreport, December 2002.

B.6.14.5a Measurement of worker exposure (Annex IIIA 7.2.3.2)

No field studies have been performed with the present Kresoxim-Methyl formulation. Such studies were not deemed necessary, since theoretical worst-case estimations demonstrated that the AOEL was not exceeded.

ALLEGRO

ALLEGRO is the commercial name for BAS 494 04 F (SC containing 125 g/L Kresoxim-Methyl and 125 g/L Epoxiconazol) BASF.

B.6.11.1b Acute oral toxicity (Annex IIIA 7.1.1.1)

- Acute oral toxicity in rats : test substance BAS 494 02 F (de Jouffrey et al.,1994, Dossier BASF)
Guidelines :
Experimental protocol in compliance with the test B.1 of Directive 92/69/EEC or Directive 84/449/EEC or OECD 401 (1981-1987) :
GLP status : yes(no attest of competent authority)
Material and methods:
5 Sprague-Dawley ICO:OFA-SD(IOPS Caw) rats/sex received a single dose of BAS 494 02 F(lot n°.93-1) in its original form at a dose level of 5000mg/kg bw.
Findings :
signs and symptoms : no effects.
mortality : no dead.
bw determination: no abnormality.
pathology : no pathologic findings.
Conclusion :
LD50 M+F> 5000 mg/kg bw

B.6.11.2b Acute dermal toxicity (Annex IIIA.7.1.2)

- Acute dermal toxicity in rats. Test substance BAS 494 02 F (de Jouffrey et al.,1994a, Dossier BASF)
Guidelines:
Protocol in compliance with method B.3 of Directive 92/69/EEC.
The study is GLP (no attest of competent authority)
Material and methods :
5 Sprague-Dawley ICO:OFA-SD(IOPS Caw) rats/sex were exposed to BAS 494 02 F (lot n°.92-5)at a dose level of 5000mg/kg bw, by dermal semi-occlusive application for 24 h.
Findings :
Mortality: no dead.
Clinical observations: no signs of systemic toxicity and no local effects.
bw gain: no abnormality.
Necropsy: no abnormalities noted.
Conclusion:
Under the conditions of this study , the LD50> 5000mg/kg bw

B.6.11.3b Acute inhalation toxicity to rats (Annex IIIA.7.1.3)

- Report: study on the acute inhalation toxicity LC50 of BAS 494 02 F as a aerosol in rats -single 4-hour exposure (Gamer and Kirsch, 1994b, Dossier BASF)
Guidelines:
Protocol in compliance with the method B.2 of the Directive 92/69/EEC.
GLP status : yes (no attest of competent authority)
Material and methods:
5 Wistar rats (CHbb:THOM(SPF))/sex were exposed for 4 h by head-nose inhalation exposure to BAS 494 02 F(B.n°.93-1) at 5.3 mg/l. The particle size distribution yielded mass median aerodynamic diameters of 2.9 µm which is in a well respirable range.(respirability : 90%)

Findings :
Mortality : no dead.
Clinical signs : all exposed animals presented an irregular and accelerated respiration during exposure.
Body weight : unchanged.
Necropsy : no abnormalities.
Conclusion : no lethality nor abnormalities were detected in the animals of the test group after exposure.
LC50 > 5.3 mg/l

B.6.11.4b Skin irritation (Annex IIIA 7.1.4)

- Acute dermal irritation in rabbits : test substance BAS 494 02 F (de Jouffrey et al.,1994b, Dossier BASF)
Guidelines:
Protocol in compliance with the test B.4 of Directive 92/69/EEC.
GLP status : yes (no attest of competent authority)
Material and methods :
6 male New Zealand White rabbits were exposed to 0.5 ml of BAS 494 02 F(lot n°.93-1) applied to the intact skin and covered with a semi-occlusive dressing during 4 h.
Findings:
Evaluation of the data, according to the EU methodology, gave the following results:

<Score erythema>$_{24+48+72\ h}$ = 0.55

<Score oedema >$_{24+48+72\ h}$ = 0

Dryness of skin was observed in 1 animal from day 3 to 5.
Conclusion :
BAS 494 02 F has no irritant properties and is therefore not classified.

B.6.11.5b Eye irritation (Annex IIIA 7.1.5)

- Acute eye irritation in rabbits : test substance BAS 494 02 F (de Jouffrey et al.,1994c, Dossier BASF)
Guidelines:
Protocol fully in compliance with the method B.5 of Directives 92/69/EEC .
GLP status :yes (no attest of competent authority)
Material and methods :
0.1 ml of BAS 494 02 F(lot n°.93-1) was applied into the conjunctival sac of the right eyelid of 6 New Zealand white rabbits.
Findings :

<Score cornea opacity>$_{24+48+72\ h}$ = 0.0

<Score iris>$_{24+48+72\ h}$ =0.0

<Score erythema>$_{24+48+72\ h}$ =0.0

<Score chemosis>$_{24+48+72\ h}$ =0.0

Conclusion :
Under the test conditions chosen BAS 494 02F does not give indication of an irritant property to the eye.

B.6.11.6b Skin sensitization (Annex IIIA 7.1.6)

- Skin sensitizing test in guinea-pigs (modified Buehler test : test substance BAS 494 02F)
(de Jouffrey et al.,1994d, Dossier BASF)
Guidelines:
Protocol not fully in compliance with the method B.6 of the Directive 92/69/EEC .
GLP status : yes (no attest of competent authority).

Material and methods :
20 Guinea-pigs (Dunkin-Hartley strain) received 9 inductions with 0.5 ml of BAS 494 02F in its original form (lot n°.93-1) during 6 h on day 1, 3, 5, 8, 10, 12, 15, 17 and 19. A challenge was carried out 10days after the third induction with 0.5 ml of the test substance formulation in its original form. A second group with 10 animals served as a control group.
Deviation from the official protocol : 9 inductions were applied instead of 3.
Findings :
During the induction period, slight erythema on day 4 in 1 animal of the treated group and dryness of the skin from day 4 to day 13 in animals of both groups, were observed.No cutaneous reaction was recorded 24 and 48 h following removal of the pads of the cutaneous challenge.
Conclusion :
ALLEGRO does not have a sensitizing effect on the skin in the modified Buehler test.

B.6.11.7b Additional studies for combinations of plant protection products (Studies as at points 7.1.1 to 7.1.6) (Annex IIIA 7.1.7)

No studies submitted

B.6.12 b Dermal absorption

B.6.12.1b Dermal absorption, *in vivo* in the rat (Annex IIIA 7.3)

-Percutaneous absorption, rat skin, Kresoxim-Methyl (BAS 490F) (formulated in propylene glycol at 0.7, 5 or 35 mg/mL), 0.007, 0.05 or 0.35 mg/cm² (Leibold, 1997)

Findings.
Only the data obtained with the animals exposed for 8h and 24h were tabulated.

8h exposure groups
The recovery of radioactivity (see table B.6.12-1) following dermal administration of [^{14}C]-BAS 490F to the rat was 95-125%. For the 24-72h exposure times, >70% of dose was recovered from the application site wash, and 2-3% from the gauze washes.The application site itself had radioactivity levels which mostly decreased as a function of post-appication time, and resulted from either unremoved residues at the surface or compound which had penetrated the skin. As there was no tape-stripping, in order to differentiate between radioactivity located in the *stratum corneum* and the underlying epidermal cells, it was considered that the skin depot should be considered potentially absorbable.

The radioactivity was excreted at about the same rate via the urine and via the faeces, and accounted for up to 1.7% (high-dose), 3.1% (middle-dose) and 10.5% (low-dose). The high urine level at 24h (10.5%) was considered 'not plausible' by the notifier, taking into account the lower values at 8h and 72h. The urinary excretion was also higher than the excretion for animals exposed during 24h (4.81% urinary and 3.24% faecal excretion). However, RMS could not find a reason to exclude this dose group, as it was not due to an outlier. Inspection of the raw data revealed an urinary level of 11.98, 11.91, 10.38 and 7.36% of the dose for the individal animals (m= 10.48±2.04%), thus the variation is acceptable. In addition the total radioactivity in excreta, tissues and carcass and application skin at 24h was about 17% after 8h exposure, and about 14% after 24h exposure, which was fairly comparable.

Notifier considered the dermal absorption complete after 24h. This opinion was not supported by the RMS, when the urinary and faecal excretion at 72h, and the decreasing application site values from 8h, 24h and 72h are considered. As in addition, the excreta+tissue+carcass values at the low dose (higher value at 24h than at 72h) were inconsistent, but remained unexplained, it was considered appropriate to consider the radioactivity of the skin depot as a potentially absorbable fraction. The average value of 24h and 72h was calculated, and an absorption value of 15.9%, 11.3% and 12.4% was obtained at the high-, middle- and low dose, respectively.

The notifier disagreed and had proposed a value of 2.9% at the high dose, and 11.4% at the low dose, based upon the sole radioactivity in the excreta+tissues+carcass.

Thus, as the tested product (dissolved in propylene glycol) did not correspond exactly to the product put on the market, and given the uncertainty on the interpretation of the 24h-72h radioactivity values, and considering that the excretion was probably not complete at 24h, RMS preferred to include the skin depot in the calculations.
As the estimated absorption rate was equivalent at all dose-levels, a conservative value of 15.91% (rounded to 16%) from the high-dose administration was considered representative for the skin absorption value.

Conclusions:
It was observed that, at 72h, the excretion of ^{14}C-BAS490F has not come to an end. Radioactivity was still present in the site of application, and could present a source of systemic exposure.
Hence, an overall absorption rate *in-vivo* in the rat of 16% was proposed for both the concentrated product and the field dilution.

Table B.6.12-1 Excretion of formulated ^{14}C-Kresoxim-Methyl after application on rat skin

Exposure time (h)	**8**			**8**			**8**			**24**		
Nominal dose (mg/cm²)	**0.35**			**0.05**			**0.007**			**0.35**	**0.05**	**0.007**
Sacrifice time (h)	**8**	**24**	**72**	**8**	**24**	**72**	**8**	**24**	**72**	**24**	**24**	**24**
Actual dose (mg/cm²)	0.38	0.36	0.36	0.053	0.051	0.053	0.0083	0.0088	0.0083	0.36	0.051	0.008
Urine	0.20	1.70	1.50	1.14	2.39	2.70	0.96	10.48	3.44	0.84	2.45	4.81
Feces	0.05	0.28	1.07	0.26	3.13	2.31	0.04	4.01	3.06	0.48	1.70	3.24
Cage wash	0.03	0.08	0.08	0.12	0.22	0.18	0.12	0.25	0.12	0.08	0.20	1.10
Blood cells	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.00	0.00	0.01
Plasma	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01
Lung	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heart	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kidneys	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.01	0.00	0.01	0.01	0.01
Liver	0.02	0.01	0.01	0.07	0.01	0.01	0.09	0.03	0.01	0.02	0.03	0.05
Carcass	0.62	0.71	0.37	2.83	0.72	0.46	2.53	1.12	0.20	1.60	1.39	3.00
excreta+tissues+carcass	*0.92*	*2.78*	*3.03*	*4.46*	*6.47*	*5.66*	*3.79*	*15.91*	*6.83*	*2.27*	*5.78*	*12.23*
Skin (application site)	14.89	22.15	9.67	4.78	8.49	1.94	3.86	1.24	0.79	11.68	1.52	2.09
Material absorbed	*15.81*	*24.93*	*12.7*	*9.24*	*14.96*	*7.6*	*7.65*	*17.15*	*7.62*	*13.95*	*7.3*	*14.32*
average 24-72h		***15.91***			***11.28***			***12.39***				
Skin (surrounding)	11.08	0.91	2.07	12.24	3.92	5.45	2.68	9.97	2.60	12.21	12.54	7.16
Protective cover	65.48	3.09	7.49	74.41	3.12	2.14	114.48	3.11	2.51	58.07	81.45	75.36
Skin wash	2.14	69.67	81.00	0.15	82.57	106.36	0.16	72.80	89.73	19.02	0.06	0.02
Total recovery	**94.51**	**98.59**	**99.93**	**96.03**	**104.29**	**117.06**	**124.96**	**103.15**	**102.54**	**106.28**	**101.34**	**96.86**

Guidelines: Protocol in compliance with test guideline OECD 417 (1984)
Remark:
(i) According to the notifier, the abnormally high radioactivity levels in the protective covers at 8h sacrifice time was due to an inappropriate work-up procedure. In contrast with the 24h- and 72h sacrifice time groups, animals were killed and put aside, without washing and removing the protective covers. As a consequence, considerable amounts of the radiolabel apparently migrated from the application site via the surrounding skin into the protective cover. By the time the protective cover was finally removed from the animals of the 8-h sacrifice groups (about 2h later), only very low amounts of radioactivity of the administered radiolabel were left on the application site skin surface. The finding was not considered to have a negative impact on the study results.
(ii) The tested product was not completely identical to the product for which authorisation was sought. BAS 494 04F is a 125 g a.s./L concentrate; in the study, the high concentration corresponds with 35 g/L. The lowest field-dilution is 1L BAS 494 04F in 400 L of water, i.e. 0.313 g a.s./L, although the recommende application volume is 1L/200L, i.e. 0.626 g a.s./L. In the study, the lowest concentration corresponds with 0.7 g a.s./L. As the dermal absorption values were estimated based upon a worst-case approach, the studies were considered valid and appropriate.

GLP status: The study is GLP
Materials and methods:

4 ♂rats/dose/time point (Wistar, Chbb:THOM (SPF)) received a dermal application (10 cm²) during 0.5h, 1h, 2h, 4h, 8h or 24h of [U-ϕ-^{14}C] Kresoxim-Methyl BAS 490F (radiochemical purity >98%, chemical purity 97.9%, B.n° 567-01, specific activity 5.136 MBq/mg), at a nominal dose level of 0.007, 0.05 or 0.35 mg/cm². Unlabelled BAS 490F had purity of 94.9% (B.n° N36). The a.i. was dissolved in propylene glycol as to obtain a formulation of 0.7, 5 or 35 mg/mL; a nominal dose volume of 100 µL/animal was provided. Animals were sacrificed at 0.5h, 1h, 2h, 4h, 8h, 24h or 72h (see table).
Disposition of animals (4 animals/point, shaded area further reported)

Duration of exposure (h)	0.5	1	2	4	8			24
Sacrifice time (h)	0.5	1	2	4	8	24	72	24

Urine and faeces and cage washes were sampled up to sacrifice time. At the respective exposure period, skin wash and bandage, gauze and silicone ring washes were collected. Blood was not collected intermittently, but at termination. Animals were sacrificed by exsanguination, and liver, kidneys, lung, heart, blood, plasma, application site, residual skin and carcass were removed or sampled. Tissues and organs were solubilised in soluene. Faeces were suspended in a.d., lyophilised and bleached (H_2O_2/isopropanol). Liquid samples, solubilised digests, and extracts were processed for LSC counting.
The study is accepted.

B.6.12.2b Comparative dermal absorption, *in vitro* using rat and human skin (Annex IIIA 7.3)
(this section was formerly located in B.5.3.3.5 –Additional observations)

-(^{14}C)-242009 : Rates of penetration through human skin and rat skin using an *in vitro* system.(Noctor and John, 1995) (Dossier BASF)
Material, methods and findings:
The rate of penetration of (^{14}C)-Reg.242009 through isolated human and rat skin preparations was assessed *in vitro* following a single application of radiolabelled formulation at 0.007, 0.050, and 0.350 mg, kresoxim-methyl/cm^2 to the epidermal surface.
Following a single application of (^{14}C)-Reg.242009 to:
-*rat skin preparations* : penetration rates of 0.475, 1.438 and 3.830 µg Reg. 242009/cm^2/h were observed at low, intermediate and high dose levels, respectively. The extrapolated lag times were 0.948, 2.091 and 1.007 h at low, intermediate and high dose levels, respectively; 70.61, 49.96 and 20.34% of the applied dose was recovered in the receptor fluid after 72 h. The overall recovery of radioactivity in these groups was 97.07, 93.14 and 101.0% of the applied dose, respectively. At the low and intermediate dose levels, the majority of the non-penetrated dose was recovered in the skin, however at the high dose level the majority of the applied dose (63.77%) was recovered in the surface washings.
-*human skin preparations* : penetration rates of 0.160, 0.935 and 1.505 µg Reg.242009 /cm^2/h were observed at low, intermediate and high dose levels, respectively; 81.86, 50.97 and 14.43% of the applied dose was recovered in the receptor fluid after 72 h. The overall recovery of radioactivity in these groups was 92.00, 90.12 and 91.08% of the applied dose, respectively. At all three dose levels, the majority of the non-penetrated dose was recovered in the surface washings.

Table B.6.12.2b: *In-vitro* dermal penetration study following a single application of (14C)-BAS 490F

species	dose (mg/cm²)	penetration rate (µg/cm²/h)	lag time (h)	recovery at 72h (% of dose)			penetration rate (% applied dose/h)	penetration in 8h (% applied dose)
				receptor fluid	skin	surface washings		
rat	0.007	0.475	0.948	70.61	25.09	1.38	6.8	54
	0.05	1.438	2.091	49.96	28.30	14.88	2.8	23
	0.35	3.830	1.007	20.34	16.84	63.77	1	9
human	0.007	0.160	3.457	81.86	4.05	6.09	2.3	18
	0.05	0.935	2.402	50.97	2.40	36.76	1.9	15
	0.35	1.505	2.474	14.43	1.55	75.10	0.4	3

Conclusion:
After single application of the compound , the rate of penetration of radioactivity through rat skin was 3.0, 1.5 and 2.5 times greater than that observed in human skin at the low, intermediate and high dose levels, respectively. In both species, rate of penetration was dose-dependent, increasing in a non-linear manner with increasing dose level.

General conclusion: estimation of the human skin absorption rate *in-vivo*

In the rat *in-vivo* skin absorption study, an overall skin absorption rate of 16% was proposed.
In the rat/human *in-vitro* skin absorption assay, it was observed that the absorption rate was about 1.5-3× higher in the rat skin than in the human skin. An overall value of 2.5× is considered reasonable.
Therefore, the estimated skin absorption value in the human was calculated as follows: 16%÷2.5 = 6.4%, rounded to 6%.
This is the value which could be used for the exposure risk assessment.

B.6.13b Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

No data, not necessary.

B.6.14b Exposure data (Annex IIIA 7.2)

BAS 490 04 F ("Allegro") is formulated as a suspension concentrate (SC) formulation containing 125 g/L Kresoxim-methyl and 125 g/L Epoxiconazole. The preparation will be used as fungicide on cereals and will be applied up to 2 times per season (21d interval). BAS 490 04 F will be used at growth stages BBCH 29-65 of the crops. The maximum application rate is 1L/ha. The recommended spray volume is 200 L/ha.

Applications of BAS 490 04 F will be performed using tractor-mounted hydrolic boom and nozzles for field applications in cereals. Therefore, the assessment of operator exposure and risk evaluation is made considering this application technique. Usage information pertinent to operator exposure is summarised in Table B.6.14.1-1b.

B.6.14.1b Estimation of operator exposure (Annex IIIA 7.2.1.1)

The dermal absorption values for respectively the neat 12.5% SC formulation BAS 490 04F ("Allegro") and the spray mix were considered 6%. The obtained predicted exposure was compared with the appropriate AOEL (0.9 mg/kg b.w./d) of Kresoxim-Methyl and expressed as % of AOEL.
No estimation was made for the a.s. Epoxiconazole (should be done at MS level).

B.6.14.1.1b Estimation of operator exposure according to UK-model

The application parameters used for the calculation in the UK-model are outlined in table B.6.14.1.1b-1.

Table B.6.14.1.1b-1: Application parameters of Kresoxim-Methyl (formulation BAS 490 04 F, "Allegro") for the UK-exposure model

Formulation		**BAS 490 04 F**
Intended uses		cereals
work rate	(ha/d)	50
application rate	(L product/ha)	1
application volume	(L spray mix/ha)	200
container size ('wide neck')*	(L)	5
application equipment		field-crop (tractor mounted hydrolic boom and nozzles)

*45 or 63 mm width

The estimated operator exposure to Kresoxim-Methyl in the UK POEM model is summarised in table B.6.14.1.1b-2 (see also details in Appendix C).

For the tractor-mounted application, the operator exposure was estimated to amount about 0.04, 0.03 or 0.0005 mg/kg b.w./d, corresponding with about 4.3%, 3.0% or 0.6% of the proposed AOEL, respectively in the absence of PPE, in the presence of PPE during mixing/loading, or in the presence of PPE in mixing/loading and application.

Table B.6.14.1.1b-2: Predicted exposure to Kresoxim-Methyl (formulation BAS 490 04 F, "Allegro") in UK-POEM (tractor-mounted hydrolic boom and nozzles)

	PPE absent	**PPE present**	
		M/L	**M/L + application**
estimated exposure (mg/kg b.w./d)	0.0391	0.0272	0.0053
% of AOEL	**4.3**	**3.0**	**0.6**

M/L: mixing and loading task

It was concluded that the estimated exposure of an operator in a tractor-mounted field spray model, was acceptable in all scenarios of application in the UK POEM model, including those where no PPE would be worn.
However, as the a.s. is classified as a Carc. Cat.3 carcinogen, PPE will be recommended anyway.

B.6.14.1.1b Estimation of operator exposure according to the German model

Table B.6.14.1.2b-1:
Application parameters of Kresoxim-Methyl (formulation BAS 490 04 F, "Allegro") in the German model

Application method		**Tractor low crops**
Formulation type		liquid
a.s. concentration (g/L)		125
Dermal absorption (%) from	**product**	6%
	spray	6%
Application rate (L/ha product)		1
Work rate/day (ha)		20
Amount a.s. (kg) handled /day		2.5

In summary, in the tractor low-crop model, the operator exposure was estimated to amount about 0.01 or 0.004 mg/kg b.w./d, corresponding with about 1% or 0.4% of the proposed AOEL, in the absence of PPE, or in the presence of PPE in mixing/loading and application, respectively (see also details in Appendix C).

Table B.6.14.1.2b-2: Predicted exposure in to Kresoxim-Methyl in the German model

	PPE absent	**PPE* present (M/L + application)**
estimated exposure (mg/kg b.w./d)	0.0096	0.0037
% of AOEL	**1.06**	**0.41**

* PPE including gloves during M/L and application

It was concluded that the estimated exposure of an operator in a tractor-mounted field-crop application, was acceptable in all scenarios in the German model, including those where no PPE would be worn.
However, as the a.s. is classified as a Carc. Cat.3 carcinogen, PPE will be recommended anyway.

General conclusion:
The handling of a 125 g/L formulation of Kresoxim-Methyl during mixing/loading and low-crop application on cereals is not expected to pose an undue risk to the operators, even when no PPE would be worn. However, as the a.s. is a Cat. 3 carcinogen, the wearing of PPE should be recommended.

B.6.14.2b Measurement of operator exposure (Annex IIIA 7.2.1.2)

No field studies have been performed with the present Kresoxim-Methyl formulation, as no exceedence of the proposed AOEL was anticipated, according to the existing operator exposure models.

B.6.14.3b Estimation of bystander exposure (Annex IIIA 7.2.2)

B.6.14.3.1b Bystander estimation according to the Lloyd and Bell model

In this calculation, the same parameters (application rate, spray volume, absorption rate, body weight) was considered as for the UK POEM operator exposure. Using these assumptions following exposure may be predicted:

Table B.6.14.3.1b: Predicted exposure of the bystander to formulation BAS 490 04 F, "Allegro" in field crop spraying, according to the model of Lloyd and Bell

ACTIVE SUBSTANCE	**Kresoxim-Methyl**	
PRODUCT	**Allegro**	
PARAMETERS		
	Dermal exposure	***Inhalation exposure***
Volume of spray solution dermally intercepted (mL)	0.1	-
Volume of spray solution intercepted by inhalation (mL/m^3)	-	0.02
Spray volume (L/ha)	200	200
Breathing rate (m^3/hour)	-	3.6
Number of hours worked/day	-	0.083
Application rate (g/ha)	125	125
Percent absorbed (%)	6	100
CALCULATIONS		
	Dermal exposure	***Inhalation exposure***
Dermal intercepted	0.00005%	
Inhalation intercepted		0.000002988%
Amount active intercepted (mg)	0.0625	0.001160180
Absorbed dose (mg)	0.00375	0.001160180
Bystander weight (kg)	60	60
Absorbed dose (mg a.s./kg bw/d)	0.0000625	0.0000186003
Total systemic (mg a.s./kg bw/d)*	0.00008111003	
AOEL (mg/kg bw/d)	0.9	
Exposure as a ratio of the AOEL:	**0.00901%**	

*: sum of dermal and inhalatory exposure, values expressed as % of AOEL

Using this model, a total (dermal+inhalatory) exposure of about 0.08 µg a.s./kg b.w./d would be expected, corresponding to about 0.009% of the AOEL.

Reference: Lloyd GA, Bell GJ, Samuels SW, Cross JV and Berrie AM, Orchard sprayers: comparative operator exposure and spray drift study, MAFF 1987 [Orchard]

B.6.14.3.2b Bystander estimation according to the Ganzelmeier model

For the calculation of the bystander exposure *(Ganzelmeier, 1995)*, worst-case application rates were supposed. The maximal value active substance per ha was considered, i.e. 0.15 kg a.s./ha.

(i) Potential dermal exposure

The bystander theoretical dermal exposure D was estimated according to the following relationship:

D = 100% deposition × drift deposition × exposed area,

where:

- the *100% deposition* equals the application rate:
= 0.125 kg a.s./ha or 12.5 mg a.s./m²

- *drift deposition*, for a 7.5 m distance in a field crop was estimated 0.13%, and,

- *exposed area* is normally estimated 0.4225 m²/person/day (exposed area includes: head, back and front of neck, forearms, $^1/_2$ upperarms and hands; working day about 6h).

However, it could be remarked that a bystander clothing would not confer a 100% protection to the spray drift. Therefore, it was considered that the remaining body area (2 – 0.4225) m²= 1.5775 m², protected by normal clothing would be exposed to 20% (default exposure mitigation factor) of the drift in a worst-case assumption.

Based upon these assumptions, the bystander dermal external exposure was:

D = (1 ×12.5 mg/m² × 0.0013 × 0.4225 m²/person/day) +(0.20 × 12.5 mg/m² × 0.0013 × 1.5775 m²/person/day)

= 0.00687 + 0.00513

= 0.0119925 mg Kresoxim-Methyl /person/day.

With a bystander bodyweight was of 60 kg, the dermal external exposure would amount to 0.000199875 mg/kg b.w./d.

Taking into account a dermal absorption of 6%, the internal exposure would calculate 0.0000119925 mg/kg b.w./d.

(ii) Potential inhalation exposure

The bystander theoretical inhalation exposure was estimated according to the following relationship:

$I = I_A \times WR \times AR$,

where:

- I_A corresponds to the specific exposure during application, estimated 0.001 mg/kg a.s./person in field crops,
- *WR* is the working rate, 20 ha/d for field crops
- *AR* is the application rate, 0.125 kg a.s./ha for Kresoxim-Methyl in "Allegro")

Thus, I = 0.001mg/kg a.s./person × 20 ha/d × 0.125 kg a.s./ha,

= 0.0025 mg / person / d

According to these values, the bystander maximal theoretical inhalation exposure was 0.0000417 mg /kg b.w./d or 0.42 µg / kg b.w./d (assuming a default body weight of 60 kg for the bystander).

If the value was adapted to a 5 minute exposure for a bystander (instead of 6h for the operator exposure, thus dividing by 360/5=72), the expected absorbed dose by inhalation would be 0.000000579 mg/kg bw, which is negligible when compared with the potential dermal exposure.

Considering the exposure about 0.0000119925 + 0.000000579 = 0.00001257 mg/kg b.w./d, about 0.0014% of the AOEL of Kresoxim-Methyl would be used up.

In conclusion, the risk for the bystander is expected to be very low.

Reference:

Ganzelmeier et al. Studies on the drift of plant protection products. Results of a test program carried out

throughout the Federal Republic of Germany. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Heft 305, 1995

B.6.14.4 Estimation of the worker exposure (Annex IIIA 7.2.2)

Kresoxim-Methyl formulated as "Allegro" (BAS 490 04F), is used as a fungicide, in cereals, and worker exposure during scouting of the field after spraying could be assessed. Therefore, a calculation was performed using the German approximation (Hoernicke et al, 1998), using the parameters as agreed for the EUROPOEM database. Further, a work duration lasting 2 h/d, and a penetration factor of 100% (assuming that PPE would not be worn in this phase) was proposed in a worst-case assumption.

The worker exposure (per kg b.w.) may be estimated by means of the following equation:
$D = DFR \times TF \times WR \times AR \times P \times A \div b.w.,$

where:
-DFR is the dislodgeable foliar residue,
-TF is the transfer factor,
-WR is the working rate,
-AR is the application rate,
-P is the penetration factor (through protective clothing),
-A is a dermal absorption of 100% (field dilution), and
- b.w. is the worker's body weight.

Assuming a DFR	= 0.003 mg a.s./cm² (Europoem II, 2002),
a transfer factor TF (high-crop estimation)	= 1000 cm²/person/h (Europoem II, 2002),
a working rate WR	= 2 h/d,
a default penetration factor P (PPE not used)	= 100%,
an application rate AR	= 0.125 kg a.s./ha
a skin absorption	= 6%, and
a body weight	= 60 kg.

D calculates as follows:

$D = 0.003 \text{ mg a.s./cm}^2 \times 1000 \text{ cm}^2\text{/person/h} \times 2 \text{ h/d} \times 0.125 \text{ kg a.s./ha} \times 1 \times 0.06 \times 1/60 \text{ kg}$
$= 0.00075 \text{ mg a.s./kg b.w./d}$

The potential dermal exposure of about 0.00075 mg/kg b.w./d, accounts for 0.083% of the AOEL.

The use of PPE would consequently further reduce the systemic exposure.

Thus, it was considered that the exposure to Kresoxim-Methyl under the current GAP, of persons entering freshly pulverised crops (for a duration of 2 h/d), would be acceptable.

References:
-Hoernicke, Nolting, Wastphal, IVA-Fachausshuss Anwenderschutz; Hinweise in der Gebrauchsanleitung zum Schutz von Personen bei Nachfolgearbeiten in mit Pflanzenschutzmitteln behandelten Kulturen (worker re-entry). Nachrichtenblatt des Deutschen Pflantzenschutzdienstes, 50, Berlin, 1998.
-The development, maintenance and dissemination of generic European databases and predictive exposure models to plant protection products. A Concerted Action under area 4 of FAIR, the Fourth Framework (Agriculture and Fisheries including Agro-Industry, Food Technology, Forestry, Aquaculture and Rural Development) specificCommunity Research and Technological Development Programme. FAIR3 CT96-1406. Finalreport, December 2002.

B.6.14.5b Measurement of worker exposure (Annex IIIA 7.2.3.2)

No field studies have been performed with the present Kresoxim-Methyl formulation. Such studies were not deemed necessary, since theoretical worst-case estimations demonstrated that the AOEL was not exceeded.

B.6.16 References relied on *[revised in March 2010]*

B.6.16.1 Active substance

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
IIA 5	Breuer J., Kieser H.	1975	The influence of feeding on clinical chemical parameters in the serum of rats *Clinical Biochemistry, 13,* Pp. 401 - 405 BASF Reg. Doc. #75/10387 (not used) No published	N	BASF
IIA 5	Weingand K. et al.	1993	Effect of overnight fasting on clinical pathology tests from rats with carbon tetrachloride induced hepatotoxicity *Toxicological Pathology, 21,* Pp. 596 BASF Reg. Doc. #93/11619 (not used) No published	N	BASF
IIA 5.1	Ecobichon,D. J.	1991	Toxic effects of pesticides. reference:in Casarett and Doull's Toxicology. The basic science of poisons. Amdur, M.O., Doull,J.and Klaassen,C.D. (eds.), chapter 8. Pergamon Press, fourth edition. No published	N	BASF
IIA 5.1	Grosshans,F.	1994	The metabolism of ^{14}CBAS 490F(^{14}C-242 009) in laying hens [redacted] BASF Reg. Doc. #94/11103 Yes unpublished	N	BASF
IIA 5.1	Grosshans, F.	1994a	The metabolism of ^{14}C-242 009 (^{14}C-BAS 490 F) in apples (incl. addendum) BASF Aktiengesellschaft, Limburgerhof, Germany BASF Reg. Doc. #94/10265, #94/10466 (addendum) Yes unpublished	N	BASF
IIA 5.1	Grosshans F.	, 1994b	The metabolism of ^{14}C-BAS 490 F (^{14}C-242 009) in wheat BASF Aktiengesellschaft, Limburgerhof, Germany BASF Reg. Doc. #94/10685 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
IIA 5.1	Grosshans,F.	1994c	The characterization of radioactive residues in wheat, beans, carrots and lettuce from a rotational crop study with [14]C-BAS 490 F ([14]C-242 009) BASF Aktiengesellschaft, Limburgerhof, Germany BASF Reg. Doc. #94/10860 Yes unpublished	N	BASF
IIA 5.1	Mayer, F.	1994	The metabolism of [[14]C]-BAS 490 F in the goat. [redacted] BASF Reg. Doc. #94/11104 Yes unpublished	N	BASF
IIA 5.1	Nelsen, J.Lewis, Ch.,Wahl,G. and Farabee D.	1995	The metabolism of [14]C-BAS 490 F in grapes. Generated by: BASF Corporation agricultural products BASF Aktiengesellschaft, Ludwigshafen, Germany BASF Reg. Doc. #95/5001 Yes unpublished	N	BASF
II A 5.1.1/1	Gans G.	1994	Study of the biokinetics of 14C-Reg.No. 242 009 (BAS 490 .. F) in rats [redacted] 1994/10992 Yes unpublished	N	BASF
II A 5.1.1/2 *[Added in March 2010]*	Gans G.	1995a	Amendment No.1 to the report: Study of the biokinetics of 14C-Reg.No. 242 009 (BAS 490 .. F) in rats [redacted] 1995/10458 Yes unpublished	Y	BASF
II A 5.1.2/1 *[Added in March 2010]*	Gans G.	1995b	Study of the tissue distribution of 14C-Reg.No. 242 009 (BAS 490 .. F) in rats [redacted] 1995/10543 Yes unpublished	Y	BASF
II A 5.1.2/2	Whitby B.R.	1993	(14C)-Reg.No. 242 009: Quantitative whole-body autoradiography following oral administration to the rat [redacted] 1993/11013 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.1.3/1	Kohl W., Dreher W.	1994	The metabolism of 14C-BAS 490 F (14C-Reg.No. 242 009) in rats [redacted] 1994/10981 Yes unpublished	N	BASF
II A 5.1.3/2 *[Added in March 2010]*	Kohl W.	1995	Addendum No. 1 to report: The metabolism of 14C-BAS 490 F (14C-Reg.No. 242 009) in rats [redacted] 1995/10366 Yes unpublished	N	BASF
II A 5.1.3/3 *[Added in March 2010]*	Kohl W.	1998	Addendum No. 2 to study code P90-M009: The metabolism of 14C-BAS 490 F (14C-Reg.No. 242 009) in rats [redacted] 1998/11159 Yes unpublished	Y	BASF
II A 5.2.1/1	Kirsch P.	1993a	Study on the acute oral toxicity of Reg.No. 242 009 in rats [redacted] 1993/10730 Yes unpublished	N	BASF
II A 5.2.2/1	Kirsch P.	1993b	Study on the acute dermal toxicity of Reg.No. 242 009 in rats [redacted] 1993/11108 Yes unpublished	N	BASF
II A 5.2.3/1	Gamer A.O.	1992	Study on the acute inhalation toxicity LC50 of Reg.No. 242 009 as a dust aerosol in rats - 4-hour exposure [redacted] 1992/10759 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.2.4/1	Rossbacher R.	1992a	Study on the acute dermal irritation/corrosion of Reg.No. 242 009 in the rabbit [redacted] 1992/11663 Yes unpublished	N	BASF
II A 5.2.5/1	Rossbacher R.	1992b	Study on the acute eye irritation of Reg.No. 242 009 in the rabbit [redacted] 1992/11664 Yes unpublished	N	BASF
II A 5.2.6/1	Rossbacher R.	1993	Report on the maximization test for the sensitizing potential of Reg.No. 242 009 in guinea pigs [redacted] 1993/10014 Yes unpublished	N	BASF
IIA 5.3	Noctor ,J.C.and John,S.A.	1995	-(14C)-242009 : Rates of penetration through human skin and rat skin using an *in vitro* system. Generated by: Corning Hazleton, Otley Road, Harrogate, North Yorkshire, England BASF Aktiengesellschaft, Ludwigshafen, Germany BASF Reg. Doc. #95/10783 Yes unpublished	N	BASF
IIA 5.3.1	Wuttke,W.	1990	Results and statement of the hormone examinations (TSH, T3, T4) of the study on the oral toxicity of Reg.n□.242 009 in Wistar rats. Administration in the diet over 4 weeks (range-finding). [redacted] BASF Reg. Doc. #92/10551 Yes unpublished	N	BASF
II A 5.3.1/1	Schilling K.	1992b	Study on the oral toxicity of Reg.No. 242 009 in Wistar rats - Administration in the diet over 4 weeks (range-finding) [redacted] 1992/10551 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.3.1/2	Schilling K.	1992a	Study on the oral toxicity of Reg.No. 242 009 in B6C3F1 mice - Administration in the diet over 4 weeks (range-finding study) [redacted] 1992/10539 Yes unpublished	N	BASF
IIA 5.3.1	Bahnemann, R.	1992	Pathology report of the study on the oral toxicity of Reg.n°.242 009 in Wistar rats. Administration in the diet over 4 weeks(range-finding). Generated by: [redacted] .SF Reg. Doc. #92/10551 Yes unpublished	N	BASF
IIA 5.3.1	Bahnemann, R.	1992a	Supplement: Pathology report of the study on the oral toxicity of Reg.n°.242 009 in B6C3F1 mice. Administration in the diet over 4 weeks(range-finding). Generated by: [redacted] .SF Reg. Doc. #92/10539 Yes unpublished	N	BASF
IIA 5.3.2	Bahnemann, R.	1994	Supplement: Pathology report Subchronic toxicity study with Reg.242 009 in Wistar rats. Administration in the diet over 3 months. Generated by: [redacted] .SF Reg. Doc. #94/10954 Yes unpublished	N	BASF
IIA 5.3.2	Bahnemann, R.	1994a	Supplement: Pathology report Subchronic toxicity study with Reg.242 009 in C57 BL mice. Administration in the diet over 3 months. Generated by: [redacted] .SF Reg. Doc. #94/10495 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
IIA 5.3.2	Bahnemann, R..	1994b	Supplement: Pathology report of subchronic toxicity study with Reg.242 009 in Beagle dogs. Administration via the diet over 3 months. Generated by: [redacted] .SF Reg. Doc. #94/10834 Yes unpublished	N	BASF
IIA 5.3.2	Bahnemann, R.,	1994c	Supplement: Pathology report on the study of the toxicity of Reg.n°.242 009 in Beagle dogs. Administration via the diet over 12 months. Generated by: [redacted] .SF Reg. Doc. #94/10832 Yes unpublished	N	BASF
II A 5.3.2/1	Mellert W., Hildebrand B.	1994d	Subchronic toxicity study with Reg.No. 242 009 in Wistar rats - Administration in the diet over 3 months [redacted] 1994/10954 Yes unpublished	N	BASF
II A 5.3.2/2	Moss D.W.	1994	Effects of Reg.No. 242 009 on enzyme levels in rat serum [redacted] 1994/10578 No unpublished	N	BASF
II A 5.3.2/3	Pickering C.E., Pickering R.G.	1978	Studies of rat alkaline phosphatase - I. Development of methods for detecting isoenzymes 1978/10526 No, not subject to GLP regulations Arch. Toxicol. 39, 249-266 (1978)	N	public
II A 5.3.2/4	Mellert W., Hildebrand B.	1995	Test study on enzyme activity after treatment with Reg.No. 242 009 in Wistar rats - Dietary administration for 3 weeks and recovery of 2 weeks [redacted] 1995/10853 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.3.2/5	Mellert W., Hildebrand B.	1994a	Subchronic oral toxicity study with Reg.No. 242 009 in C 57 BL mice - Administration in the diet for 3 months [redacted] 1994/10495 Yes unpublished	N	BASF
II A 5.3.2/6	Mellert W., Hildebrand B.	1994c	Amendment No. 1 to the report: Subchronic oral toxicity study with Reg.No. 242 009 in C57BL mice - Administration in the diet for 3 months [redacted] 1994/10942 Yes unpublished	Y	BASF
II A 5.3.3/1	Mellert W., Hildebrand B.	1994b	Report on the study of the toxicity of Reg.No. 242 009 in beagle dogs - Administration via the diet over 3 months [redacted] 1994/10834 Yes unpublished	N	BASF
II A 5.3.4/1	Hellwig J.	1994	Report on the study of the toxicity of Reg.No. 242 009 in beagle dogs - Administration via the diet over 12 months [redacted] 1994/10832 Yes unpublished	N	BASF
II A 5.3.7/1	Kirsch P., Hildebrand B.	1994	Study of the dermal toxicity of Reg.No. 242 009 in Wistar rats - Application to the intact skin over 3 weeks (21 applications) [redacted] 1994/11070 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.4.1/1	Engelhardt G., Hoffmann H.D.	1993a	Report on the study of Reg.No. 242 009 (ZST test substance No.: 91/180) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1993/10249 Yes unpublished	N	BASF
II A 5.4.1/2	Engelhardt G., Hildebrand B.	1994	Report on the study of Reg.No. 242 009 (ZHT test substance No.: 91/180-2) in the Ames test (Salmonella/Mammalian-Microsome mutagenicity test - Standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1994/10753 Yes unpublished	N	BASF
II A 5.4.2/1	Hoffmann H.D., Engelhardt G.	1993	In vitro cytogenetic investigations of Reg.No.: 242 009 in human lymphocytes BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1993/10493 Yes unpublished	N	BASF
II A 5.4.3/1	Poelloth C., Hoffmann H.D.	1994a	Gene mutation test in chinese hamster ovary cells (HPRT Locus Assay) with Reg.No. 242 009 BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1994/10350 Yes unpublished	N	BASF
II A 5.4.3/2	Poelloth C., Hoffmann H.D.	1994b	In vitro - Unscheduled DNA Synthesis (UDS) assay in rat hepatocytes with Reg.No. 242 009 BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1994/10351 Yes unpublished	N	BASF
II A 5.4.4/1	Engelhardt G., Hoffmann H.D.	1993b	Cytogenetic study *in vivo* of Reg.No. 242 009 in mice - Micronucleus test - Single intraperitoneal administration [redacted] 1993/11104 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.4.4/2 *[Added in March 2010]*	Hoffmann H.D., Engelhardt G.	1997	Cytogenetic study in vivo with Reg.No. 242 009 in the rat micronucleus test - Single intraperitoneal administration [redacted] 1997/10312 Yes unpublished	Y	BASF
II A 5.4.5/1	Poelloth C., Hoffmann H.D.	1994c	Ex vivo - unscheduled DNA synthesis (UDS) assay and s-phase-response in rat hepatocytes with Reg.No. 242 009 [redacted] 1994/10867 Yes unpublished	N	BASF
II A 5.4.5/2	Poelloth C., Hoffmann H.D.	1994d	Ex vivo - Unscheduled DNA synthesis (UDS) assay in rat hepatocytes with Reg.No. 242 009 after administration in the diet for 3 weeks [redacted] 1994/10894 Yes unpublished	N	BASF
II A 5.4.6/1 *[Added in March 2010]*	Honarvar N.	2002	Chromosome aberration assay in mouse spermatogonial cells with Reg.No. 242 009 - Application: once, orally [redacted] 2002/1011621 Yes unpublished	Y	BASF
II A 5.4.6/2 *[Added in March 2010]*	Ma L., Leibold E.	2003	Analytical report: Stability analysis of BAS 490 F in corn oil BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2003/1028919 Yes unpublished	Y	BASF
II A 5.5.1/1	Mellert W.	1994a	Chronic toxicity study with Reg.No. 242 009 in Wistar rats - Administration in the diet for 24 months [redacted] 1994/10951 Yes unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.5.1/2 *[Added in March 2010]*	Hildebrandt P.K.	1995b	Pathology working group (PWG) report compound Reg.No. 242 009 - 24 month chronic toxicity study (project No. 70C0180/91007) in Wistar rats Pathco Inc.; Ijamsville MD; United States of America 1995/10979 No unpublished	Y	BASF
IIA 5.5.1	Pappritz,G.,	1994	Supplement: Pathology report [redacted] BASF Reg. Doc. #94/10951 Yes unpublished	N	BASF
IIA 5.5.2	Pappritz,G.,	1994a	Supplement: Pathology report [redacted] BASF Reg. Doc. #94/10953 Yes unpublished	N	BASF
II A 5.5.2/1	Mellert W.	1994b	Carcinogenicity study with Reg.No. 242 009 in Wistar rats - Administration in the diet for 24 months [redacted] 1994/10953 Yes unpublished	N	BASF
II A 5.5.2/2 *[Added in March 2010]*	Hildebrandt P.K.	1995a	Pathology working group (PWG) report compound Reg.No. 242 009 - 24-month carcinogenicity study (project No. 70C0180/91006) in Wistar rats Pathco Inc.; Ijamsville MD; United States of America 1995/10977 No unpublished	Y	BASF
II A 5.5.2/3 *[Added in March 2010]*	Hildebrandt P.K.	1995c	Pathology working group (PWG) report compound Reg.No. 242 009 - 24-month chronic toxicity (project No. 70C0180/91007) and carcinogenicity (project No. 70C0180/91006) studies in Wistar rats Pathco Inc.; Ijamsville MD; United States of America 1995/10980 No unpublished	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.5.2/4 *[Added in March 2010]*	Kamp H. et al.	2008	BAS 490 F (Kresoxim-methyl): Carcinogenicity study in Wistar rats - CrlGlxBrlHan:Wl - Administration via the diet over 24 months [redacted] 2008/1028083 Yes unpublished	Y	BASF
II A 5.5.3/1	Mellert W., Gelbke H.-P.	1994	Carcinogenicity study with Reg.No. 242 009 in C57BL mice - Administration in the diet for 18 months [redacted] 1994/10919 Yes unpublished	N	BASF
II A 5.5.4/1	Pölloth C.	1994a	S-phase-response study with Reg.No. 242 009 in Wistar rats after administration in the diet for 3 weeks [redacted] 1994/10922 Yes unpublished	N	BASF
II A 5.5.4/2	Pölloth C.	1994b	S-phase response study with Reg.No. 242 009 in 16-month old Wistar rats after administration in the diet for 3 weeks [redacted] 1994/10984 Yes unpublished	N	BASF
II A 5.5.4/3	Mellert W.et al.	1996	Reg.No. 242 009 - S-phase response study in male Wistar rats including reversibility - Administration in the diet up to 13 weeks [redacted] 1996/10053 Yes unpublished	N	BASF
II A 5.5.4/4 *[Added in March 2010]*	Mellert W.	1997	S-phase-response study with BAS 490 02 F (Reg.No. 242 009) in Wistar rats after administration in the diet for 3 weeks (supplementary study to project No. 83M0180/910149) [redacted] 1997/10318 Yes unpublished	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.5.4/5	Gamer A.O., Hildebrand B.	1995	Study of foci initiating activity of Reg.No. 242 009 in Wistar rats [redacted] 1995/10244 Yes unpublished	N	BASF
II A 5.5.4/6 *[Added in March 2010]*	Harada T.	1997	BAS 490 F (Reg.No. 242 009): Medium-term promotion hepatocarcinogenesis study in rats [redacted] 1997/10918 No unpublished	Y	BASF
II A 5.5.4/7	Mellert W. et al.	1995a	Reg.No. 242 009 - Electron microscopic examinations of liver samples to assess mitochondria from old Wistar rats treated for 3 weeks in the diet [redacted] 1995/11105 Yes unpublished	N	BASF
II A 5.5.4/8	Mellert W. et al.	1995b	Reg.No. 242 009 - Electron microscopic examinations of liver samples to assess peroxisomes from Wistar rats treated for 3 weeks in the diet [redacted] 1995/11106 Yes unpublished	N	BASF
II A 5.5.4/9 *[Added in March 2010]*	Mellert W. et al.	1996a	Reg.No. 242 009 - Examination of enzyme activities in the liver of Wistar rats - Administration in the diet for 3 weeks [redacted] 1996/10100 Yes unpublished	Y	BASF
IIA 5.5.5	Van Ravenzwaay, B	1996	Kresoxim-methyl: mechanism and assessment of liver tumour induction BASF Reg. Doc. #96/10078 No unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.6.1/1	Hellwig J., Gelbke H.-P.	1994b	Reproduction toxicity study with Reg.No. 242 009 in Wistar rats - Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation) [redacted] 1994/10950 Yes unpublished	N	BASF
II A 5.6.10/1	Hellwig J., Gelbke H.-P.	1994a	Study of the prenatal toxicity of Reg.No. 242 009 in Wistar rats after oral administration (gavage) [redacted] 1994/10833 Yes unpublished	N	BASF
II A 5.6.11/1	Hellwig J., Hildebrand B.	1993	Study of the prenatal toxicity of Reg.No. 242 009 in rabbits after oral administration (gavage) [redacted] 1993/10980 Yes unpublished	N	BASF
II A 5.7.1/1 *[Added in March 2010]*	Mellert W. et al.	1996c	Reg.No. 242 009 - Acute oral neurotoxicity study in Wistar rats [redacted] 1996/10410 Yes unpublished	Y	BASF
II A 5.7.4/1 *[Added in March 2010]*	Mellert W. et al.	1996d	Reg.No. 242 009 - Subchronic oral neurotoxicity study in Wistar rats - Administration in the diet for 3 months [redacted] 1996/10411 Yes unpublished	Y	BASF
IIA 5.8	Lucier, G.W.	1992	Receptor mediated carcinogenesis. In: Mechanisms of Carcinogenesis in risk Identification. Ed. Vainio,H., Magee,P.N., McGregor, B.N. and McMichael,A.J. Lyon, International Agency for Research on Cancer, pp 87-112 No published	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.8.2	Dubach-Powell J.R. et al.	1994	Effects on the vital functions of animals - General pharmacology of - Reg.No. 242 009 [redacted] 1994/11219 Yes unpublished	N	BASF
II A 5.8.2	Mellert W. et al.	1996b	Kresoxim-methyl (Reg.No. 242 009): Pilot study - Dietary administration to rats after repeated pretreatment with Dimethoate [redacted] 1996/10246 No unpublished	N	BASF
II A 5.8/1 *[Added in March 2010]*	Kirsch P.	1995	Study on the acute oral toxicity of Reg.No. 262 451 in rats [redacted] 1995/10213 Yes unpublished	Y	BASF
II A 5.8/2 *[Added in March 2010]*	Engelhardt G.	1995b	Report on the study of Reg.No. 262 451 (ZHT test substance No. 94/520) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1995/10409 Yes unpublished	Y	BASF
II A 5.8/3 *[Added in March 2010]*	Kirsch P.	1994b	Study on the acute oral toxicity of Reg.No. 291 685 in rats [redacted] 1994/11172 Yes unpublished	Y	BASF
II A 5.8/4 *[Added in March 2010]*	Engelhardt G., Hoffmann H.D.	1995a	Study of Reg.No. 291 685 (ZHT test substance No.: 94/262) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli / Mammalian-microsome reverse mutation assay (standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1995/10027 Yes unpublished	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
II A 5.8/5 *[Added in March 2010]*	Engelhardt G., Hoffmann H.D.	1995b	Amendment No.1 to study of Reg.No. 291 685 (ZHT test substance No. 94/262) in Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1995/10482 Yes unpublished	Y	BASF
II A 5.8/6 *[Added in March 2010]*	Kirsch P.	1994a	Study on the acute oral toxicity of Reg.No. 292 932 in rats [redacted] 1994/11171 Yes unpublished	Y	BASF
II A 5.8/7 *[Added in March 2010]*	Engelhardt G.	1995a	Study of Reg.No. 292 932 (ZHT test substance No.: 94/263) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1995/10026 Yes unpublished	Y	BASF
II A 5.8/8 *[Added in March 2010]*	Engelhardt G.	1996	Report on the study of Reg.No. 339 774, BF 490-15 (ZHT test substance No.: 96/28) in the Ames Salmonella/Mammalian-microsome mutagenicity test and Escherichia coli/Mammalian-microsome reverse mutation assay (standard plate test and preincubation test) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1996/10353 Yes unpublished	Y	BASF
II A 5.9.1/1 *[Added in March 2010]*	Zober A. et al.	1997	Assessment of liver function and clinical outcomes in employees having direct contact with Kresoxim-methyl BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1997/10111 No, not subject to GLP regulations unpublished	N	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
5.14.1.a	Pesticides residues in food	1994	Report of the joint meeting of the FAO panel of experts on pesticide Residues in food and environment and the WHO expert group on pesticides residues.77-99 No published	N	BASF

B.6.16.2 Plant protection products ALLEGRO (BAS 494 02 F) and BAS 494 04 F

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
III A 7.1/1 *[Added in March 2010]*	Stinchcombe S.	2008	BAS 494 04 F - Evaluation of the toxicological significance of the formulation composition change from BAS 494 02 F to BAS 494 04 F - Minor change reasoning BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep. 2008/1026404 No, not subject to GLP regulations unpublished		BASF
III A 7.1.1/1	Jouffrey S. de et al.	1994a	Acute oral toxicity in rats: BAS 494 02 F [redacted] 1994/11131 Yes unpublished		BASF
III A 7.1.2/1	Jouffrey S. de et al.	1994b	Acute dermal toxicity in rats: BAS 494 02 F [redacted] 1994/11132 Yes unpublished		BASF
III A 7.1.3/1	Gamer A.O., Kirsch P.	1994	Study on the acute inhalation toxicity LC50 of BAS 494 02 F as a liquid aerosol in rats - 4-hour exposure [redacted] 1994/11051 Yes unpublished		BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
III A 7.1.4/1	Jouffrey S. de et al.	1994c	BAS 494 02 F: Acute dermal irritation in rabbits [redacted] 1994/11133 Yes unpublished		BASF
III A 7.1.5/1	Jouffrey S. de et al.	1994d	Acute eye irritation in rabbits: BAS 494 02 F [redacted] 1994/11135 Yes unpublished		BASF
III A 7.1.6/1	Jouffrey S. de et al.	1994e	BAS 494 02 F: Skin sensitization test in guinea-pigs (modified Buehler test: 9 applications) [redacted] 1994/11675 Yes unpublished		BASF
III A 7.5.1/1 *[Added in March 2010]*	Evans J. et al.	2000c	Policy # 003.1 - Science Advisory Council for Exposure - Agricultural transfer coefficients 2000/1023421 No, not subject to GLP regulations United States Environmental Protection Agency		public
III A 7.6.1/1 *[Added in March 2010]*	Leibold E. et al.	1997	14C-BAS 490 F - Study of the dermal resorption in rats [redacted] 1997/10217 Yes unpublished		BASF
III A 7.6.2/1	John S.A., Noctor J.C.	1995	(14C)-242 009: Rates of penetration through human skin and rat skin using an in vitro system Corning Hazleton; Harrogate North Yorkshire HG3 1PY; United Kingdom 1995/10783 Yes unpublished		BASF
III A 7.6.2/2 *[Added in March 2010]*	Gamer A.O., Landsiedel R.	2008	14C-BAS 480 F (Epoxiconazole) in BAS 494 04 F - Study of penetration through human skin in vitro BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep. 2008/1004823 Yes unpublished		BASF

B.6.16.3 Plant protection product CANDIT (BAS 490 02 F)

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
III A 7.1.1/1	Kirsch P., Hildebrand B.	1994a	Study on the acute oral toxicity of BAS 490 02 F in rats [redacted] 1994/10294 Yes unpublished		BASF
III A 7.1.2/1	Kirsch P., Hildebrand B.	1994b	Study on the acute dermal toxicity of BAS 490 02 F in rats [redacted] 1994/10295 Yes unpublished		BASF
III A 7.1.3/1 *[Added in March 2010]*	Gamer A.O., Kirsch P.	1994	Study on the acute inhalation toxicity LC50 of BAS 490 02 F as a dust aerosol in rats - 4-hour exposure [redacted] 1994/10061 Yes unpublished		BASF
III A 7.1.4/1	Rossbacher R., Kirsch P.	1994a	Study on the acute dermal irritation/corrosion of BAS 490 02 F in the rabbit [redacted] 1994/10107 Yes unpublished		BASF
III A 7.1.5/1	Rossbacher R., Kirsch P.	1994b	Study on the acute eye irritation of BAS 490 02 F in the rabbit [redacted] 1994/10108 Yes unpublished		BASF
III A 7.1.6/1	Rossbacher R., Kirsch P.	1994c	Report on the Buehler test for the sensitizing potential of BAS 490 02 F in guinea pigs [redacted] 1994/10296 Yes unpublished		BASF
III A 7.5.1/1 *[Added in March 2010]*	Evans J. et al.	2000	Policy # 003.1 - Science Advisory Council for Exposure - Agricultural transfer coefficients 2000/1023421 No, not subject to GLP regulations [redacted]		public

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
III A 7.6.2/1 *[Added in March 2010]*	Gamer A.O., Landsiedel R.	2008	14C-BAS 490 F (Kresoxim-methyl) in BAS 490 02 F - Study of penetration through human skin in vitro BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1020044 Yes unpublished		BASF

ANNEX B

Kresoxim-methyl

Appendix C : Estimation of the exposure

A. BAS 490 02 F ('Candit', Kresoxim-Methyl 50% WG)

1. UK POEM exposure model

Tractor drawn air assisted orchard sprayer 500 l/ha model; no gloves

PRODUCT DATA				
Product	BAS 490 02 F			
Active substance	KRESOXIM-METHYL			
Concentration	500	mg/g		
Formulation type	WDG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use a.s. concentration	1	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	1	kg		
Hand contamination/operation	0,01	ml		
Application dose	0,3	kg product/ha		
Work rate	15	ha/day		
Number of operations	5	/day		
Hand contamination	0,05	g/day		
Protective clothing	**none**			
Transmission to skin	100	%		
Dermal exposure to formulation	0,05	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique - tractor drawn orchard sprayer with hydraulic nozzles				
Application volume	150	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	**none**	permeable	permeable	
Penetration	100	2	5	%
Dermal exposure	10	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121,2	ml/day		
ABSORBED DOSE	Mix/load		Application	
Dermal exposure	0,05	g/day	121,2	ml/day
Concentration of a.s.	500	mg/ml	1	mg/ml
Dermal exposure to a.s.	25	mg/day	121,2	mg/day
Percent absorbed	0,3	%	9	%
Absorbed dose	0,075	mg/day	10,908	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0,05	ml/h		
Duration of exposure	6	h		
Concentration of a.s.	1	mg/ml		
Inhalational exposure to a.s.	0,3	mg/day		
Percent absorbed	100	%		
Absorbed dose	0,3	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	11,283	mg/day		
Operator body weight	60	kg		
Operator exposure	0,18805	mg/kg bw/day		
AOEL	0,9	mg/kg bw/day		
% of AOEL	**20,9%**			

Tractor drawn air assisted orchard sprayer 500 l/ha model; gloves during M/L

PRODUCT DATA				
Product	BAS 490 02 F			
Active substance	KRESOXIM-METHYL			
Concentration	500	mg/g		
Formulation type	WDG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use a.s. concentration	1	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	1	kg		
Hand contamination/operation	0,01	ml		
Application dose	0,3	kg product/ha		
Work rate	15	ha/day		
Number of operations	5	/day		
Hand contamination	0,05	g/day		
Protective clothing	**gloves**			
Transmission to skin	1	%		
Dermal exposure to formulation	0,0005	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique - tractor drawn orchard sprayer with hydraulic nozzles				
Application volume	150	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	**none**	permeable	permeable	
Penetration	100	2	5	%
Dermal exposure	10	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121,2	ml/day		
ABSORBED DOSE	Mix/load		Application	
Dermal exposure	0,0005	g/day	121,2	ml/day
Concentration of a.s.	500	mg/ml	1	mg/ml
Dermal exposure to a.s.	25	mg/day	121,2	mg/day
Percent absorbed	0,3	%	9	%
Absorbed dose	0,00075	mg/day	10,908	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0,05	ml/h		
Duration of exposure	6	h		
Concentration of a.s.	1	mg/ml		
Inhalational exposure to a.s.	0,3	mg/day		
Percent absorbed	100	%		
Absorbed dose	0,3	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	11,20875	mg/day		
Operator body weight	60	kg		
Operator exposure	0,18686125	mg/kg bw/day		
AOEL	0,9	mg/kg bw/day		
% of AOEL	**20,8%**			

Tractor drawn air assisted orchard sprayer 500 l/ha model; gloves during M/L and application

PRODUCT DATA				
Product	BAS 490 02 F			
Active substance	KRESOXIM-METHYL			
Concentration	500	mg/g		
Formulation type	WDG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use a.s. concentration	1	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	1	kg		
Hand contamination/operation	0,01	ml		
Application dose	0,3	kg product/ha		
Work rate	15	ha/day		
Number of operations	5	/day		
Hand contamination	0,05	g/day		
Protective clothing	**gloves**			
Transmission to skin	1	%		
Dermal exposure to formulation	0,0005	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique - tractor drawn orchard sprayer with hydraulic nozzles				
Application volume	150	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	**gloves**	permeable	permeable	
Penetration	10	2	5	%
Dermal exposure	4	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85,2	ml/day		
ABSORBED DOSE	Mix/load		Application	
Dermal exposure	0,0005	g/day	85,2	ml/day
Concentration of a.s.	500	mg/ml	1	mg/ml
Dermal exposure to a.s.	25	mg/day	85,2	mg/day
Percent absorbed	0,3	%	9	%
Absorbed dose	0,00075	mg/day	7,668	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0,05	ml/h		
Duration of exposure	6	h		
Concentration of a.s.	1	mg/ml		
Inhalational exposure to a.s.	0,3	mg/day		
Percent absorbed	100	%		
Absorbed dose	0,3	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	7,96875	mg/day		
Operator body weight	60	kg		
Operator exposure	0,1328125	mg/kg bw/day		
AOEL	0,9	mg/kg bw/day		
% of AOEL	**14,8%**			

Low level hydraulic knapsack sprayer model (15 litre spray tank); no gloves

PRODUCT DATA					
Product	BAS 490 02 F				
Active substance	KRESOXIM-METHYL				
Concentration	500	mg/g			
Formulation type	WDG				
Main solvent					
Concentration of solvent		w/w			
Maximum in-use a.s. concentration	1	mg/ml			
EXPOSURE DURING MIXING AND LOADING					
Container size	1	kg			
Hand contamination/operation	0,01	ml			
Application dose	0,3	kg product/ha			
Work rate	1	ha/day			
Number of operations	10	/day			
Hand contamination	0,1	g/day			
Protective clothing	**none**				
Transmission to skin	100	%			
Dermal exposure to formulation	0,1	g/day			
EXPOSURE DURING SPRAY APPLICATION					
Application technique - knapsack, hydraulic nozzle, low level					
Application volume	150	spray/ha			
Volume of surface contamination	50	ml/h			
Distribution	Hands		Trunk	Legs	
	25		25	50	%
Clothing	**none**		permeable	permeable	
Penetration	100		20	18	%
Dermal exposure	10		2,5	4,5	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	102	ml/day			
ABSORBED DOSE	Mix/load			Application	
Dermal exposure	0,1	g/day		102	ml/day
Concentration of a.s.	500	mg/ml		1	mg/ml
Dermal exposure to a.s.	50	mg/day		102	mg/day
Percent absorbed	0,3	%		9	%
Absorbed dose	0,15	mg/day		9,18	mg/day
INHALATION EXPOSURE DURING SPRAYING					
Inhalation exposure	0,02	ml/h			
Duration of exposure	6	h			
Concentration of a.s.	1	mg/ml			
Inhalational exposure to a.s.	0,12	mg/day			
Percent absorbed	100	%			
Absorbed dose	0,12	mg/day			
PREDICTED EXPOSURE					
Total absorbed dose	9,45	mg/day			
Operator body weight	60	kg			
Operator exposure	0,1575	mg/kg bw/day			
AOEL	0,9	mg/kg bw/day			
% of AOEL	**18%**				

Low level hydraulic knapsack sprayer model (15 litre spray tank); gloves during M/L

PRODUCT DATA					
Product	BAS 490 02 F				
Active substance	KRESOXIM-METHYL				
Concentration	500	mg/g			
Formulation type	WDG				
Main solvent					
Concentration of solvent		w/w			
Maximum in-use a.s. concentration	1	mg/ml			
EXPOSURE DURING MIXING AND LOADING					
Container size	1	kg			
Hand contamination/operation	0,01	ml			
Application dose	0,3	kg product/ha			
Work rate	1	ha/day			
Number of operations	10	/day			
Hand contamination	0,1	g/day			
Protective clothing	**gloves**				
Transmission to skin	1	%			
Dermal exposure to formulation	0,001	g/day			
EXPOSURE DURING SPRAY APPLICATION					
Application technique - knapsack, hydraulic nozzle, low level					
Application volume	150	spray/ha			
Volume of surface contamination	50	ml/h			
Distribution	Hands		Trunk	Legs	
	25		25	50	%
Clothing	**none**		permeable	permeable	
Penetration	100		20	18	%
Dermal exposure	10		2,5	4,5	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	102	ml/day			
ABSORBED DOSE	Mix/load			Application	
Dermal exposure	0,001	g/day		102	ml/day
Concentration of a.s.	500	mg/ml		1	mg/ml
Dermal exposure to a.s.	0,5	mg/day		102	mg/day
Percent absorbed	0,3	%		9	%
Absorbed dose	0,0015	mg/day		9,18	mg/day
INHALATION EXPOSURE DURING SPRAYING					
Inhalation exposure	0,02	ml/h			
Duration of exposure	6	h			
Concentration of a.s.	1	mg/ml			
Inhalational exposure to a.s.	0,12	mg/day			
Percent absorbed	100	%			
Absorbed dose	0,12	mg/day			
PREDICTED EXPOSURE					
Total absorbed dose	9,30	mg/day			
Operator body weight	60	kg			
Operator exposure	0,155025	mg/kg bw/day			
AOEL	0,9	mg/kg bw/day			
% of AOEL	**17%**				

Low level hydraulic knapsack sprayer model (15 litre spray tank); gloves during M/L and application

PRODUCT DATA					
Product	BAS 490 02 F				
Active substance	KRESOXIM-METHYL				
Concentration	500	mg/g			
Formulation type	WDG				
Main solvent					
Concentration of solvent		w/w			
Maximum in-use a.s. concentration	1	mg/ml			
EXPOSURE DURING MIXING AND LOADING					
Container size	1	kg			
Hand contamination/operation	0,01	ml			
Application dose	0,3	kg product/ha			
Work rate	1	ha/day			
Number of operations	10	/day			
Hand contamination	0,1	g/day			
Protective clothing	**gloves**				
Transmission to skin	1	%			
Dermal exposure to formulation	0,001	g/day			
EXPOSURE DURING SPRAY APPLICATION					
Application technique - knapsack, hydraulic nozzle, low level					
Application volume	150	spray/ha			
Volume of surface contamination	50	ml/h			
Distribution	Hands		Trunk	Legs	
	25		25	50	%
Clothing	**gloves**		permeable	permeable	
Penetration	10		20	18	%
Dermal exposure	1,25		2,5	4,5	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	49,5	ml/day			
ABSORBED DOSE	Mix/load			Application	
Dermal exposure	0,001	g/day		49,5	ml/day
Concentration of a.s.	500	mg/ml		1	mg/ml
Dermal exposure to a.s.	0,5	mg/day		49,5	mg/day
Percent absorbed	0,3	%		9	%
Absorbed dose	0,0015	mg/day		4,455	mg/day
INHALATION EXPOSURE DURING SPRAYING					
Inhalation exposure	0,02	ml/h			
Duration of exposure	6	h			
Concentration of a.s.	1	mg/ml			
Inhalational exposure to a.s.	0,12	mg/day			
Percent absorbed	100	%			
Absorbed dose	0,12	mg/day			
PREDICTED EXPOSURE					
Total absorbed dose	4,5765	mg/day			
Operator body weight	60	kg			
Operator exposure	0,076275	mg/kg bw/day			
AOEL	0,9	mg/kg bw/day			
% of AOEL	**8%**				

2. German exposure model

Tractor high crops

Use information

Product	BAS 490 02F		Active substance	KRESOXIM-METHYL	
	WG				
Formulation type	Tractor high crops		A.s. concentration	500	mg/g
Method of use			Dose (product)	0,3	kg product/ha
Work rate	20	ha/day	Dose (a.s.)	0,15	kg a.s./ha
			Amount handled	3	kg a.s./day

Exposures - mix/loading

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,008	mg/kg a.s. handled	0,024	mg a.s./day	Gloves	0,024	mg a.s./day
Dermal - hands	2	mg/kg a.s. handled	6	mg a.s./day		0,06	mg a.s./day

Exposures - application

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,018	mg/kg a.s. handled	0,054	mg a.s./day	None	0,054	mg a.s./day
Dermal - head	1,2	mg/kg a.s. handled	3,6	mg a.s./day	Gloves	3,6	mg a.s./day
Dermal - hands	0,7	mg/kg a.s. handled	2,1	mg a.s./day	Coverall + boots	0,021	mg a.s./day
Dermal - body	9,6	mg/kg a.s. handled	28,8	mg a.s./day		1,44	mg a.s./day

Total exposures	Estimated exposures		% absorbed	Estimated exposure (PPE)		% absorbed
Total potential inhalation	0,078	mg a.s./day	100%	0,078	mg a.s./day	100%
Total dermal - mix	6	mg a.s./day	0,30%	0,06	mg a.s./day	0,30%
Total dermal - application	34,5	mg a.s./day	9,0%	5,061	mg a.s./day	9,0%
Total absorbed dose	3,201	mg a.s./day		0,53367	mg a.s./day	
Body weight	70	kg		70	kg	
mg/kg bw/day	0,0457286	mg a.s./kg bw/day		0,0076239	mg a.s./kg bw/day	
AOEL 0,9 mg a.s./kg bw/day	**5,08%**			**0,85%**		

Hand-held high crops

Use information

Product	BAS 490 02F		Active substance	KRESOXIM-METHYL	
	WG				
Formulation type	Hand high crops		A.s. concentration	500	mg/g
Method of use			Dose (product)	0,3	kg product/ha
Work rate	1	ha/day	Dose (a.s.)	0,15	kg a.s./ha
			Amount handled	0,15	kg a.s./day

Exposures - mix/loading

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,02	mg/kg a.s. handled	0,003	mg a.s./day	Gloves	0,003	mg a.s./day
Dermal - hands	21	mg/kg a.s. handled	3,15	mg a.s./day		0,0315	mg a.s./day

Exposures - application

	Specific exposures		Estimated exposures		None	Estimated exposure (PPE)	
					None		
Inhalation	0,3	mg/kg a.s. handled	0,045	mg a.s./day	Gloves	0,045	mg a.s./day
Dermal - head	4,8	mg/kg a.s. handled	0,72	mg a.s./day	Coverall + boots	0,72	mg a.s./day
Dermal - hands	10,6	mg/kg a.s. handled	1,59	mg a.s./day		0,0159	mg a.s./day
Dermal - body	25	mg/kg a.s. handled	3,75	mg a.s./day		0,1875	mg a.s./day

Total exposures	Estimated exposures		% absorbed	Estimated exposure (PPE)		% absorbed
Total potential inhalation	0,048	mg a.s./day	100%	0,048	mg a.s./day	100%
Total dermal - mix	3,15	mg a.s./day	0,30%	0,0315	mg a.s./day	0,30%
Total dermal - application	6,06	mg a.s./day	9,0%	0,9234	mg a.s./day	9,0%
Total absorbed dose	0,60285	mg a.s./day		0,1312005	mg a.s./day	
Body weight	70	kg		70	kg	
mg/kg bw/day	0,0086121	mg a.s./kg bw/day		0,0018743	mg a.s./kg bw/day	
AOEL 0,9 mg a.s./kg bw/day	**0,96%**			**0,21%**		

B. BAS 490 04 F ('Allegro, Kresoxim-Methyl 12.5% SC)

1. UK POEM exposure model

Tractor mounted hydraulic boom and nozzles model; no gloves

PRODUCT DATA					
Product	BAS 490 04 F				
Active substance	KRESOXIM-METHYL				
Concentration	125	mg/g			
Formulation type	SC				
Main solvent					
Concentration of solvent		w/w			
Maximum in-use a.s. concentration	0.625	mg/ml			
EXPOSURE DURING MIXING AND LOADING					
Container size	5	l			
Hand contamination/operation	0.01	ml			
Application dose	1	l product/ha			
Work rate	50	ha/day			
Number of operations	10	/day			
Hand contamination	0.1	ml/day			
Protective clothing	**none**				
Transmission to skin	100	%			
Dermal exposure to formulation	0.1	ml/day			
EXPOSURE DURING SPRAY APPLICATION					
Application technique - tractor, hydraulic boom and nozzles					
Application volume	200	spray/ha			
Volume of surface contamination	10	ml/h			
Distribution	Hands		Trunk	Legs	
	65		10	25	%
Clothing	**none**		permeable	permeable	
Penetration	100		5	15	%
Dermal exposure	6.5		0.05	0.375	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	41.55	ml/day			
ABSORBED DOSE	Mix/load			Application	
Dermal exposure	0.1	ml/day		41.55	ml/day
Concentration of a.s.	125	mg/ml		0.625	mg/ml
Dermal exposure to a.s.	12.5	mg/day		25.96875	mg/day
Percent absorbed	6	%		6	%
Absorbed dose	0.75	mg/day		1.558125	mg/day
INHALATION EXPOSURE DURING SPRAYING					
Inhalation exposure	0.01	ml/h			
Duration of exposure	6	h			
Concentration of a.s.	0.625	mg/ml			
Inhalational exposure to a.s.	0.0375	mg/day			
Percent absorbed	100	%			
Absorbed dose	0.0375	mg/day			
PREDICTED EXPOSURE					
Total absorbed dose	2.345625	mg/day			
Operator body weight	60	kg			
Operator exposure	0.03909375	mg/kg bw/day			
AOEL	0,9	mg/kg bw/day			
% of AOEL	**4.3%**				

Tractor mounted hydraulic boom and nozzles model; gloves during M/L

PRODUCT DATA					
Product	BAS 490 04 F				
Active substance	KRESOXIM-METHYL				
Concentration	125	mg/g			
Formulation type	SC				
Main solvent					
Concentration of solvent		w/w			
Maximum in-use a.s. concentration	0.625	mg/ml			
EXPOSURE DURING MIXING AND LOADING					
Container size	5	l			
Hand contamination/operation	0.01	ml			
Application dose	1	l product/ha			
Work rate	50	ha/day			
Number of operations	10	/day			
Hand contamination	0.1	ml/day			
Protective clothing	**gloves**				
Transmission to skin	5	%			
Dermal exposure to formulation	0.005	ml/day			
EXPOSURE DURING SPRAY APPLICATION					
Application technique - tractor, hydraulic boom and nozzles					
Application volume	200	spray/ha			
Volume of surface contamination	10	ml/h			
Distribution	Hands		Trunk	Legs	
	65		10	25	%
Clothing	**none**		permeable	permeable	
Penetration	100		5	15	%
Dermal exposure	6.5		0.05	0.375	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	41.55	ml/day			
ABSORBED DOSE	Mix/load			Application	
Dermal exposure	0.005	ml/day		41.55	ml/day
Concentration of a.s.	125	mg/ml		0.625	mg/ml
Dermal exposure to a.s.	0.625	mg/day		25.96875	mg/day
Percent absorbed	6	%		6	%
Absorbed dose	0.0375	mg/day		1.558125	mg/day
INHALATION EXPOSURE DURING SPRAYING					
Inhalation exposure	0.01	ml/h			
Duration of exposure	6	h			
Concentration of a.s.	0.625	mg/ml			
Inhalational exposure to a.s.	0.0375	mg/day			
Percent absorbed	100	%			
Absorbed dose	0.0375	mg/day			
PREDICTED EXPOSURE					
Total absorbed dose	1.633125	mg/day			
Operator body weight	60	kg			
Operator exposure	0.02721875	mg/kg bw/day			
AOEL	0,9	mg/kg bw/day			
% of AOEL	**3.0%**				

Tractor mounted hydraulic boom and nozzles model; gloves during M/L and application

PRODUCT DATA					
Product	BAS 490 04 F				
Active substance	KRESOXIM-METHYL				
Concentration	125	mg/g			
Formulation type	SC				
Main solvent					
Concentration of solvent		w/w			
Maximum in-use a.s. concentration	0.625	mg/ml			
EXPOSURE DURING MIXING AND LOADING					
Container size	5	l			
Hand contamination/operation	0.01	ml			
Application dose	1	l product/ha			
Work rate	50	ha/day			
Number of operations	10	/day			
Hand contamination	0.1	ml/day			
Protective clothing	**gloves**				
Transmission to skin	5	%			
Dermal exposure to formulation	0.005	ml/day			
EXPOSURE DURING SPRAY APPLICATION					
Application technique - tractor, hydraulic boom and nozzles					
Application volume	200	spray/ha			
Volume of surface contamination	10	ml/h			
Distribution	Hands		Trunk	Legs	
	65		10	25	%
Clothing	**gloves**		permeable	permeable	
Penetration	10		5	15	%
Dermal exposure	0.65		0.05	0.375	ml/h
Duration of exposure	6	h			
Total dermal exposure to spray	6.45	ml/day			
ABSORBED DOSE	Mix/load			Application	
Dermal exposure	0.005	ml/day		6.45	ml/day
Concentration of a.s.	125	mg/ml		0.625	mg/ml
Dermal exposure to a.s.	0.625	mg/day		4.03125	mg/day
Percent absorbed	63	%		63	%
Absorbed dose	0.0375	mg/day		0.241875	mg/day
INHALATION EXPOSURE DURING SPRAYING					
Inhalation exposure	0.01	ml/h			
Duration of exposure	6	h			
Concentration of a.s.	0.625	mg/ml			
Inhalational exposure to a.s.	0.0375	mg/day			
Percent absorbed	100	%			
Absorbed dose	0.0375	mg/day			
PREDICTED EXPOSURE					
Total absorbed dose	0.316875	mg/day			
Operator body weight	60	kg			
Operator exposure	0.00528125	mg/kg bw/day			
AOEL	0,9	mg/kg bw/day			
% of AOEL	**0.6%**				

2. German exposure model

Tractor field crops

Use information

Product	BAS 490 04F		Active substance	KRESOXIM-METHYL	
Formulation type	Liquid		A.s. concentration	125	mg/ml
Method of use	Tractor field crops		Dose (product)	1	litres product/ha
Work rate	20	ha/day	Dose (a.s.)	0,125	kg a.s./ha
			Amount handled	2,5	kg a.s./day

Exposures - mix/loading

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,0006	mg/kg a.s. handled	0,0015	mg a.s./day	Gloves	0,0015	mg a.s./day
Dermal - hands	2,4	mg/kg a.s. handled	6	mg a.s./day		0,06	mg a.s./day

Exposures - application

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,001	mg/kg a.s. handled	0,0025	mg a.s./day	None	0,0025	mg a.s./day
Dermal - head	0,06	mg/kg a.s. handled	0,15	mg a.s./day	None	0,15	mg a.s./day
Dermal - hands	0,38	mg/kg a.s. handled	0,95	mg a.s./day	None	0,95	mg a.s./day
Dermal - body	1,6	mg/kg a.s. handled	4	mg a.s./day		4	mg a.s./day

Total exposures	Estimated exposures		Percent absorbed	Estimated exposure (PPE)		Percent absorbed
Total potential inhalation	0,004	mg a.s./day	100%	0,004	mg a.s./day	100%
Total dermal - mix	6	mg a.s./day	6,00%	0,06	mg a.s./day	6,00%
Total dermal - application	5,1	mg a.s./day	6,0%	5,1	mg a.s./day	6,0%
Total absorbed dose	0,67	mg a.s./day		0,3136	mg a.s./day	
Body weight	70	kg		70	kg	
mg/kg bw/day	0.0095714	mg a.s./kg bw/day		0,00448	mg a.s./kg bw/day	
AOEL	0,9	mg a.s./kg bw/day	**1.06%**			**0,50%**

Tractor field crop

Use information

Product	BAS 490 04F		Active substance	KRESOXIM-METHYL	
	Liquid				
Formulation type	Tractor field crops		A.s. concentration	125	mg/ml
Method of use			Dose (product)	1	litres product/ha
Work rate	20	ha/day	Dose (a.s.)	0,125	kg a.s./ha
			Amount handled	2,5	kg a.s./day

Exposures - mix/loading

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,0006	mg/kg a.s. handled	0,0015	mg a.s./day	Gloves	0,0015	mg a.s./day
Dermal - hands	2,4	mg/kg a.s. handled	6	mg a.s./day		0,06	mg a.s./day

Exposures - application

	Specific exposures		Estimated exposures		PPE (including RPE)	Estimated exposure (PPE)	
					None		
Inhalation	0,001	mg/kg a.s. handled	0,0025	mg a.s./day	None	0,0025	mg a.s./day
Dermal - head	0,06	mg/kg a.s. handled	0,15	mg a.s./day	Gloves	0,15	mg a.s./day
Dermal - hands	0,38	mg/kg a.s. handled	0,95	mg a.s./day	None	0,0095	mg a.s./day
Dermal - body	1,6	mg/kg a.s. handled	4	mg a.s./day		4	mg a.s./day

Total exposures	Estimated exposures		Percent absorbed	Estimated exposure (PPE)		
Total potential inhalation	0,004	mg a.s./day	100%	0,004	mg a.s./day	100%
Total dermal - mix	6	mg a.s./day	6,00%	0,06	mg a.s./day	6,00%
Total dermal - application	5,1	mg a.s./day	6,0%	4,1595	mg a.s./day	6,0%
Total absorbed dose	0,67	mg a.s./day		0,25717	mg a.s./day	
Body weight	70	kg		70	kg	
mg/kg bw/day	0,0095714	mg a.s./kg bw/day		0,0036739	mg a.s./kg bw/day	
AOEL	0,9	mg a.s./kg bw/day	**1,06%**			**0,41%**