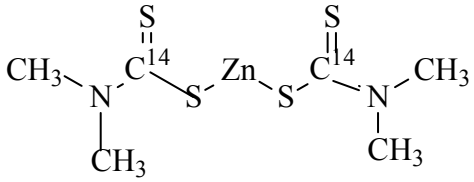
Revised version: Ziram – 1/09/2000 completed on: July 2002

ANNEX B

Ziram

B.6 Toxicology and metabolism

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



When the draft monograph was prepared, information on metabolism and identification of metabolites was lacking.
This point was addressed to the notifier and a preliminary study on ziram absorption, distribution and excretion following oral administration to the rat was provided in may 2000:

(^{14}C)-Ziram: ADME in the rat following single oral administration (Covance study n° 514/40, Study status report, 2000).

Findings:
According to the results reported in table B.6.1. -1, absorption rate of ziram is low: peak plasma concentrations were attained after 6.8-10 h for the low dose reaching 24 h after high dose.
Increasing the dose by a factor of 10, increased Cmax plasma 17 times and Cmax blood 30 times; simultaneously, the half-life of elimination increased with the dose, all together suggesting increased saturation of excretion.
Elimination half-live is higher than 24 h suggesting that accumulation may occur. Binding to red blood cells is suggested.

Table B.6.1-1: kinetic parameters of ziram after single low and high dose in rat.

	15 mg/kg bw				**150 mg/kg bw**			
	M		**F**		**M**		**F**	
	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma
C_{max} (µg equiv./g)	1.286	0.859	1.304	0.804	31.31	13.68	36.06	17.98
T_{max}(h)	14.4	6.8	21.6	10.4	43.2	24	48	24
$T_{1/2el}$ (h)	170.5	33.44	196.2	38.9	234.1	36.1	247.9	38.22
$AUC_{(0-t)}$(µg equi.h/g)	157.0	21.97	194.2	22.24	4721	659.2	5498	818.2
$AUC_{(0-\infty)}$(µg equiv./g)	273.8	29.95	357.5	31.31	11064	809.4	13181	994.5

Tissue distribution:

Tissue distribution was large:
2 hr after single oral low dose, high radioactivity was detected in carcass and fat. Excretory organs such as liver, kidney and lung reached a maximum at 8 hr while thyroid contained the highest radioactivity at 24 h.
2 h after single oral high dose, highest levels of radioactivity were observed in carcass (male and females), and lung (females); at 8 hr, radioactivity residue was the highest in female pituitary gland while liver and kidney and other tissues contained the highest residue at 24h. At that time, blood and plasma residues were also the highest. Female presented higher levels of radioactivity in tissues than males.
At 96 h, radioactivity was still present in all tissues except brain, fat and pituitary for males and fat for females. Highest residues were observed in thyroid, liver, kidney and blood. It was not possible to estimate from these results how many percent or µg of the initial administered dose were recovered in the organs (Table B.6.1-2).

Table B.6.1. -2: Tissue distribution in rats after low and high dose of ziram

	µg equivalents/g (^{14}C)-ziram															
	2 h				**8 h**				**24 h**				**96 h**			
	15 mg		150mg		15 mg		150mg		15 mg		150mg		15 mg		150mg	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
car cass	6.7	7.2	130	**134**	2.9	2.0	81	95	.4	.3	10	21	.14	.17	1.5	2.0
Bone	.15	.15	1.6	1.1	.55	.33	3.2	2.6	.20	.15	4.9	5.6	.15	.15	2.0	2.2
Lung	.93	1.14	5.8	**51**	1.6	1.7	9.2	6.5	.84	.87	9.5	20	.39	.51	3.0	4.9
Liver	3.1	2.4	17	13	4.6	4.2	11	13	2.4	2.2	14	21	.84	.98	4.5	6.0
Kidney	2.3	1.5	7.4	5.9	3.8	2.7	9.8	9.1	1.4	1.2	19	38	0.6	0.7	4.0	6.9
Brain	.43	.34	1.8	2.6	.70	.69	2.8	3.3	.21	.21	4.5	9.8	.1	.12	-	.98
Fat	2.4	2.1	10	10	1.9	2.0	19	12	.54	.26	11	39	.35	.34	-	-
Spleen	1.2	1.1	3.8	9.3	1.4	1.4	5.6	4.6	0.7	0.6	8.8	18	.37	.43	2.1	4.2
Pituitary	.25	.11	.51	-	.80	-	2.7	1.02	.48	.21	2.8	26	.19	.23	-	1.7
Testes/ Ovary	.43	.48	1.7	1.8	.80	.15	2.9	3.8	.25	.03	5.3	12	.09	.01	.54	2.2
Thyroid	.37	1.1	3.2	62	1.8	3.6	7.5	2.8	2.5	4.7	21	24	.63	-	4.8	9.4
Adrenal	.95	.85	2.8	5.6	2.7	1.9	5.7	7.2	1.2	1.3	9.1	17	.49	.73	2.2	4.5
Muscle	.35	.31	2.0	1.0	.66	.54	2.3	1.9	.22	.19	3.9	8.3	.11	.12	.99	.46
Heart	.63	.49	2.2	3.4	1.2	1.1	3.9	4.3	.75	.73	8	15	.52	.56	3.2	5.5
Blood	.55	.50	1.8	1.5	1.6	1.5	5	6.2	1.0	1.0	9	16	.69	.89	2.9	7.8
Plasma	.68	.57	2.1	1.7	1.23	1.08	4.2	4.1	.34	.31	5.6	10	.08	.13	1.2	1.5
Total	**21.4**	**20.3**	**193.7**	**303.9**	**28.2**	**24.9**	**170.8**	**177.4**	**13.4**	**13.36**	**146.4**	**300.7**	**5.74**	**6.08**	**32.9**	**60.2**

- : Not detected

Conclusion: from the preliminary results here reported, it can be concluded that absorption rate and extent are low; elimination rate is very low suggesting accumulation of ziram or its metabolites. Tissue distribution is large and comparable in male and female rats after low dose. After single high dose or repeated low dose, female rats, had higher levels of radioactivity at 2 hr, in thyroid , lung and adrenals.
Maximal tissue residue is observed at 8 h after low dose and at 24h after high dose.
At 96-h post dosing, radioactivity was still present in the majority of organs.

Guidelines: Results are described as a preliminary report.
In this study, the applicant explained that they had problems for achieving a mass balance and the excretion study was therefore, delayed. In addition, a number of pilot studies have been conducted both to assist with the study design and also to solve problems associated with the excretion balance studies.
Originally the low and high dose levels were set at 15 and 352 mg/kg bw respectively, however during the early stages of the study, rats showed toxic effects at the high dose level. The high dose level was therefore lowered to 150 mg/kg bw and the high dose pharmacokinetic study were repeated. There were also problems for obtaining a homogenous suspension and the formulation method will be used.

Material and methods: 5 Sprague Dawley rats/sex/dose received a single oral dose of 15 or 150-mg/kg bw ziram (100 µCi/kg; radiochemical purity > 98%; non-radiolabelled ziram, batch n°.; >98%) in 0.3% (w/v) aqueous carboxymethyl cellulose, for the 168 h toxicokinetic study. At 168-h post dose, animals were killed and blood and tissues were collected.
Bile duct of 4 rats/sex/dose was cannulated.
Information on the extent of metabolism will be undertaken from a pilot study. Samples from volatile traps may also be taken to identify volatile metabolites.

22/08/2001 Main study:

^{14}C-ziram: absorption, distribution, metabolism and excretion following oral administration to the rat (Hardwick and Lappin, 2001)

Findings:

Absorption:

As suggested by Tmax, absorption of ziram is slow and slows down with increased dose. Ziram binds at a slower rate (T_{max}blood >T_{max} plasma) but more intensively to blood than plasma constituents (C_{max}blood >C_{max}plasma). $T_{1/2elim}$ in blood is longer than in plasma leading to a 10 times higher AUC in blood than in plasma.
Increasing the dose by a factor of 10 is associated with a more than 10 times increase in AUC, a 10 times increase in Cmax, while Tmax and T1/2elim are increased but not proportional to the dose, suggesting that, by increasing the dose, absorption is slower but more important/or that elimination is retarded. Slight differences are apparent between male and female rats suggesting that ziram reaches more important concentrations in females than in males.
Overall, $T_{1/2el}$ is higher than 24 h suggesting that accumulation may occur after repeated exposure (Table B.6.1-3).
Bile represents only a very minor route of excretion (table B.6.1-4).
Based on the results of exhaled volatiles as well as urinary excretion, absorption is estimated to reach 59% within 72 h after a single low dose exposure (table B.6.1-8). Absorption increases up to 81.2% after repeated low dose exposure.
In the monograph, it was concluded that the oral absorbed dose reached 58-61% after low dose and a correction factor of 50% was applied. Therefore, based on the new data, no further correction is necessary.

Table B.6.1-3: kinetic parameters of ziram after single low and high dose in rat.

	15 mg/kg bw				**150 mg/kg bw**			
	M		F		M		F	
	Blood	**Plasma**	**Blood**	**Plasma**	**Blood**	**Plasma**	**Blood**	**Plasma**
C_{max} (µg equiv./g)	1.286	0.859	1.304	0.804	12.88	6.548	15	8.373
T_{max}(h)	14.4	6.8	21.6	10.4	33.6	24	43.2	24
$T_{1/2el}$ (h)	170.5	33.44	196.2	38.9	170.6	57.59	234.2	65.4
$AUC_{(0-t)}$(µg equi.h/g)	157.0	21.97	194.2	22.24	1821	296.1	2348	401.9
$AUC_{(0-\infty)}$(µg equiv./g)	273.8	29.95	357.5	31.31	3215	419.4	5177	558.1

Table B.6.1-4: Biliary excretion of radioactivity after a single low or high dose ziram

	% Of administered dose	
Dose:	**50 mg/kg bw**	**100 mg/kg bw**
	M	**M**
Urine 48 h	16.86	9.584
Faeces 48 h	1.669	3.120
Bile 1h	Nd	0.171
4h	0.597	0.374
6h	0.260	0.067
12h	0.305	0.354
24h	0.875	0.596
48h	0.176	0.377
Total	2.213	1.939
Cage wash 48 h	2.464	1.314
Cage debris 48 h	Nd	Nd
Total 48 h	23.20	15.96

Nd: not detected

Distribution:

Distribution and uptake of radioactivity by tissues was rapid, with all tissues being exposed within 2 h after dosing (concentrations were <0.1% of administered dose except for the tissues reported in table B.6.1-5). Maximum concentrations of radioactivity were achieved in most tissues at 8h post-dose administration in the low dose group and at 24 h in the high dose group. Maximum concentrations of radioactivity were not proportional with respect to dose level.

High levels of radioactivity were absorbed in the carcass within 2 h but these levels fell rapidly with time. The highest levels of radioactivity were detected in the organs of metabolism and excretion and after 168 h there were only low levels of radioactivity in all tissues. High levels were also detected in highly vascularised tissues resulting probably from the strong binding capacity of CS_2 to cross-link proteins (such as spectrin in erythrocytes).

Table B.6.1-5 Tissue distribution of radioactive material (concentration> 0.1% of dose) in male and female rats after oral administration of ^{14}-C ziram as a function of time.

	Percent of administered dose					
Dose	**15 mg/kg bw**		**14 x 15 mg/kg bw /d**		**150 mg/kg bw**	
	M	F	M	F	M	F
Carcass 2 h	**37.88**	**40.30**	-	-	**73.93**	**74.82**
8h	15.50	11.52	-	-	45.22	52.73
24h	1.79	1.439	-	-	5.502	11.45
96h	0.714	0.741	-	-	0.914	1.106
168h	1.048	1.085	0.814	0.791	0.831	0.976
Liver 2 h	0.902	0.584	-	-	0.421	0.320
8h	1.215	1.026	-	-	0.279	0.297
24h	0.681	0.527	-	-	0.361	0.526
96h	0.261	0.224	-	-	0.126	0.141
14 days			0.142	0.117		
Kidney 2h	0.121	0.076			0.035	0.030
8h	0.187	0.152			0.045	0.042
24h	0.076	0.063			0.088	0.189
96h	0.034	0.036			0.020	0.032
14 days			0.031	0.033		
Lung 2h						0.181
8h						0.019
24h						0.063
96h						0.016

Metabolism:

Approximately 52-65% of the administered dose was metabolised from which 43-59% were identified (Table B.6.1-6).

The most important fraction of ziram was metabolised into volatile compounds excreted in air. These volatile metabolites, collectively accounted for ca 51% of the administered dose, and consisted of CS_2, (COS) and CO_2. Increasing the dose enhances slightly formation of these metabolites (Table B.6.1-6).
In urine, independently of the dose, 3 main metabolites were detected, metabolites 4, 6 and 8. Two minor urinary metabolites were identified: dimethylamine-thiazolidine carboxylic acid (M1) and S-glucuronide (5) of dimethyldithiocarbamic acid. In faeces, low levels of thiram were detected (Table B.6.1-7).

Ziram is rapidly reduced to dithiocarbamic acid with loss of the metal ion (fig B.6.1-1). Traces of thiram were detected in faeces (0.08-0.18%). Dimethylthiocarbamic acid is further decomposed to dimethylamine and CS_2 or conjugated:

- Decomposition of dimethyldithiocarbamate to CS_2 is the principal route. CS_2 is oxidised to CO_2 and COS (by the mixed function oxidase system). Degradation of ziram via this route seems to increase with the dose. Total radioactivity excreted via air represents 42 to 65% of the dose (table B.6.1-6). As suggested in the open literature (Graham et al, CRC in toxicology, 1995, 25, 91-112) CS_2 interacts directly with amines, sulfhydryls and hydroxyl functions by nucleophilic addition (not shown). Reaction of CS_2 with cysteinyl sulfhydryls of cysteine or glutathione yields trithiocarbamates, which can cyclize to form thiazolidine-2-thione-4- carboxylic acid (TTCA; U1). Urine samples were analysed for the detection of TTCA but the analysis was not successful due to technical problems. However, the standard of thiazolidine-2-thione-4-carboxylic acid eluted with a retention time of 14.2 min and radioactivity from a urine sample in this region accounted for 0.02% of administered dose. So, if thiazolidine-2-thione-4- carboxylic acid (U1) was present, it would not have exceeded this percentage.
- Dimethyldithiocarbamic acid is conjugated with glucuronic acid and glutathione giving rise to urinary metabolites. These conjugation routes are minor pathways as identified glucuronides represent 1.5-5.4% of the dose excreted in urine. Conjugation of dimethyldithiocarbamic acid or ziram directly with GSH would be catabolized to the cysteine conjugate via the cysteinyl-glycine conjugate which then cyclise, looses H_2S to form 2-dimethylamine-thiazolidine carboxylic acid or M-1 (0.45-1.3% in urine) (fig B.6.1-1).

Table B.6.1-6: Identified metabolites after oral administration of C^{14} ziram in rats

	Percentage of the administered dose					
	15 mg/kg bw		**14 x 15 mg/kg bw /d**		**150 mg/kg bw**	
Urine	M	F	M	F	M	F
M1	0.87	0.81	0.85	1.28	0.82	0.45
5*	0.9	0.32	0.41	0.15	0.45	0.31
Faeces						
Thiram	0	0.18	0.08	0	0	0.15
Expired air						
CO_2	5.84	9.15	6.55	9.41	3.54	9.416
CS_2	36.26	33.26	41.79	48.53	52.3	56.429
Total expired	42.1	42.41	48.34	57.94	55.84	65.845
Total identified	**43.87**	**43.72**	**49.68**	**59.37**	**57.11**	**66.755**
Total identified + unidentified	**52.9**	**53.47**	**63.12**	**72.8**	**64.92**	**64.94**

M1: dimethylamine-thiazolidine carboxylic acid
5 * S-glucuronide of dimethyldithiocarbamic acid

Table B.6.1-7: Identification of metabolites in rat urine (0-48h) and faeces after oral administration of ziram.

	Percentage of the administered dose											
	15 mg/kg bw				**14 x 15 mg/kg bw /d**				**150 mg/kg bw**			
	Urine		**Faeces**		**Urine**		**Faeces**		**Urine**		**Faeces**	
	M	F	M	F	M	F	M	F	M	F	M	F
1	0	0	0.41	0.15	0	0	0.41	0.61	0	0	0.17	0.42
2	0	0	0	0	0	0	0	0	0	0	0.52	0
M1	0.87	0.81	0	0	0.85	1.28	0	0	0.82	0.45	0	0
4	**2.5**	**2.88**	0	0	**2.62**	**2.48**	0	0	**1.61**	**2.16**	0.61	0
5*	0.9	0.32	0	0	0.41	0.15	0	0	0.45	0.31	0	0
6	**3.0**	**2.82**	0	0.2	**5.48**	**4.91**	0.29	0.65	**2.42**	**1.56**	0.48	0.11
7	0.87	1.03	0	0	0.98	0.85	0	0	0.65	0.61	0	0
Thiram	0	0	0	0.18	0	0	0.08	0	0	0	0	0.15
8	**2.2**	**2.66**	0	0	**3.74**	**3.96**	0	0	**1.98**	**1.23**	0	0
Total %	**10.34**	**10.5**	**0.4**	**0.53**	**14.08**	**13.6**	**0.78**	**1.26**	**7.93**	**6.32**	**1.78**	**0.68**

M1: dimethylamine-thiazolidine carboxylic acid
5 * S-glucuronide of dimethyldithiocarbamic acid

Excretion:

Following oral administration, the majority of radioactivity was excreted as volatiles (CS_2, CO_2) in expired air (36-50%) within 24 h, reaching 42 to 60% of the dose within 168h. Excretion via urine and faeces reaches 16 % and 3% respectively. Increasing the dose 10 times decreased slightly urinary excretion but increased exhalation via CS_2. After repeated exposure, urinary excretion was slightly increased (21%) as well as CS_2 exhalation (42-50%). Overall, within 168 h, 64 to 85% of the administered dose was excreted (table B.6.1-8 and table B.6.1-9). The overall recovery in these experiments was lower than would normally be expected due to difficulty in trapping the high levels of volatiles.

Table B.6.1-8: excretion of ziram after single low, repeated low, and high dose in rat as a function of time.

	Cumulative excretion as a function of dose (%)					
	15 mg/kg bw		**14 x 15 mg/kg bw /d**		**150 mg/kg bw**	
	M	F	M	F	M	F
Urine						
6 h	6.997	8.085	10.29	9.997	3.121	2.426
24h	14.85	14.49	19.79	19.35	10.80	8.063
48h	15.53	15.62	20.32	19.98	12.67	10.21
72h	15.76	15.91	20.52	20.22	12.96	10.59
168 h	16.14	16.37	20.81	20.57	13.25	10.89
Cage wash						
6 h	No sample	No sample	No sample	No sample	No sample	No sample
24 h	0.496	0.719	0.390	0.905	1.008	0.977
48 h	0.567	0.875	0.443	1.033	1.131	1.123
72 h	0.567	0.925	0.461	1.058	1.159	1.161
168 h	0.567	0.936	0.493	1.123	1.182	1.187
Expired air						
Trap1-24 h	3.167	5.918	3.287	5.893	1.789	1.938
-72 h	3.534	6.435	3.599	6.268	2.142	2.310
Trap 2- 24 h	2.163	2.431	2.852	2.980	1.260	1.060
- 72 h	2.304	2.720	2.950	3.148	1.405	1.216
Trap 3- 24 h	30.06	26.00	33.25	41.53	40.09	39.55
-72h	34.56	30.00	39.47	48.35	52.16	53.68
Trap 4-24h	0.914	2.496	2.215	Not detected	0.012	1.670
-72h	1.703	3.262	2.325	0.184	0.146	2.749

Σ Exp.air: trap 1,2,3,4 24 h	36.3	36.8	41.6	50.4	43.15	44.2
Σ Exp.air: trap 1,2,3,4 72 h	42.1	42.4	48.3	58	55.8	60
Total absorbed dose: Σ urine+cage wash + expired air at:						
24h	51.64	52.00	61.8	70.78	55.08	53.2
72h	58.42	59.24	69.33	79.2	69.9	71.7
Faeces						
6 h	No sample	No sample	No sample	No sample	No sample	No sample
24 h	2.12	2.056	2.589	3.581	3.454	1.665
48 h	2.801	2.59	2.806	3.875	4.313	2.451
72 h	2.916	3.056	2.961	4.005	4.464	2.717
168 h	3.068	3.238	3.112	4.145	4.574	2.844
Total excreted dose: Σ urine + cage wash + expired air + faeces at:						
24h	53.8	54.06	64.4	74.4	58.5	54.8
72 h	61.33	62.3	72.3	83.2	74.4	74.4

Trap1: CO_2, trap 2: CO_2, trap 3: CS_2; trap 4: CS_2

Table B.6.1-9: Recovery of ^{14}C radioactivity 168 h (except for exhaled dose measured up to 72 h) after oral administration of ziram.

	% Of administered dose					
	15 mg/kg bw		**14 x 15 mg/kg bw /d**		**150 mg/kg bw**	
	M	F	M	F	M	F
Urine	16.14	16.37	20.81	20.57	13.25	10.89
Faeces	3.068	3.238	3.112	4.145	4.574	2.844
Cage wash	0.567	0.936	0.493	1.123	1.182	1.187
CO_2	3.534+2.304	6.435+2.72	3.599+2.95	6.268+3.148	2.142+1.405	2.310+1.216
CS_2	34.56+1.703	30+3.262	39.47+2.325	48.35+0.184	52.16+0.146	53.68+2.749
Exhaled dose	42.1	42.4	48.3	58.0	55.9	60.0
Cage debris	0.002	0.238	0.289	0.129	ND	0.377
Carcass	1.048	1.085	0.814	0.791	0.831	0.976
Tissues	0.346	0.337	0.230	0.213	0.190	0.232
Total	**63.27**	**64.62**	**74.09**	**84.93**	**75.88**	**76.50**

ND: not detected

Guidelines: experimental protocol of the study in compliance with the test method B dir. 87/302/EEC.
Comment: overall recovery of radioactivity in these studies was lower than would normally be expected due to difficulty in trapping the high level of volatile (ca 70%). For collection of excreta at defined times, cages had to be opened and rinsed, having as consequence that recovery are somewhat low. Exhaustive attempts were made to achieve quantitative recoveries including at various stages, the use of volatile trapping systems consisting of 1M KOH, 2 ethoxyethanol, athanediol, viles reagent, H2SO4, Zinsser oxysolve, modified Viles reagent and activated charcoal. The suitability of various trapping systems was then investigated. The extensive attempts resulted in recoveries rising from around 45% in the first experiments to 80% in the last. Nevertheless, recoveries on the main experiments were in the region of 63-85%.
Material and methods: 5 Sprague Dawley (Crl: CD BR) rats/sex/dose received a single oral dose by gavage of 15 or 150 mg/kg bw ziram (184.2 µCi/mg for batch n° 1140A and 139.8µCi/mg for batch n° 1278A-4)-radiochemical purity 95.6 and 96.3% respectively diluted with non-radiolabelled ziram, batch n°. V743/G7705992; 98.99 %) in 0.3% (w/v) aqueous carboxymethyl cellulose. Bile canulated rats received 50 mg/kg bw and were sacrificed after 48 h. Repeated dosed rats received 15 mg/kg bw/d.
Information on the extent of metabolism will be undertaken from a pilot study. Samples from volatile traps may also be taken to identify volatile metabolites.

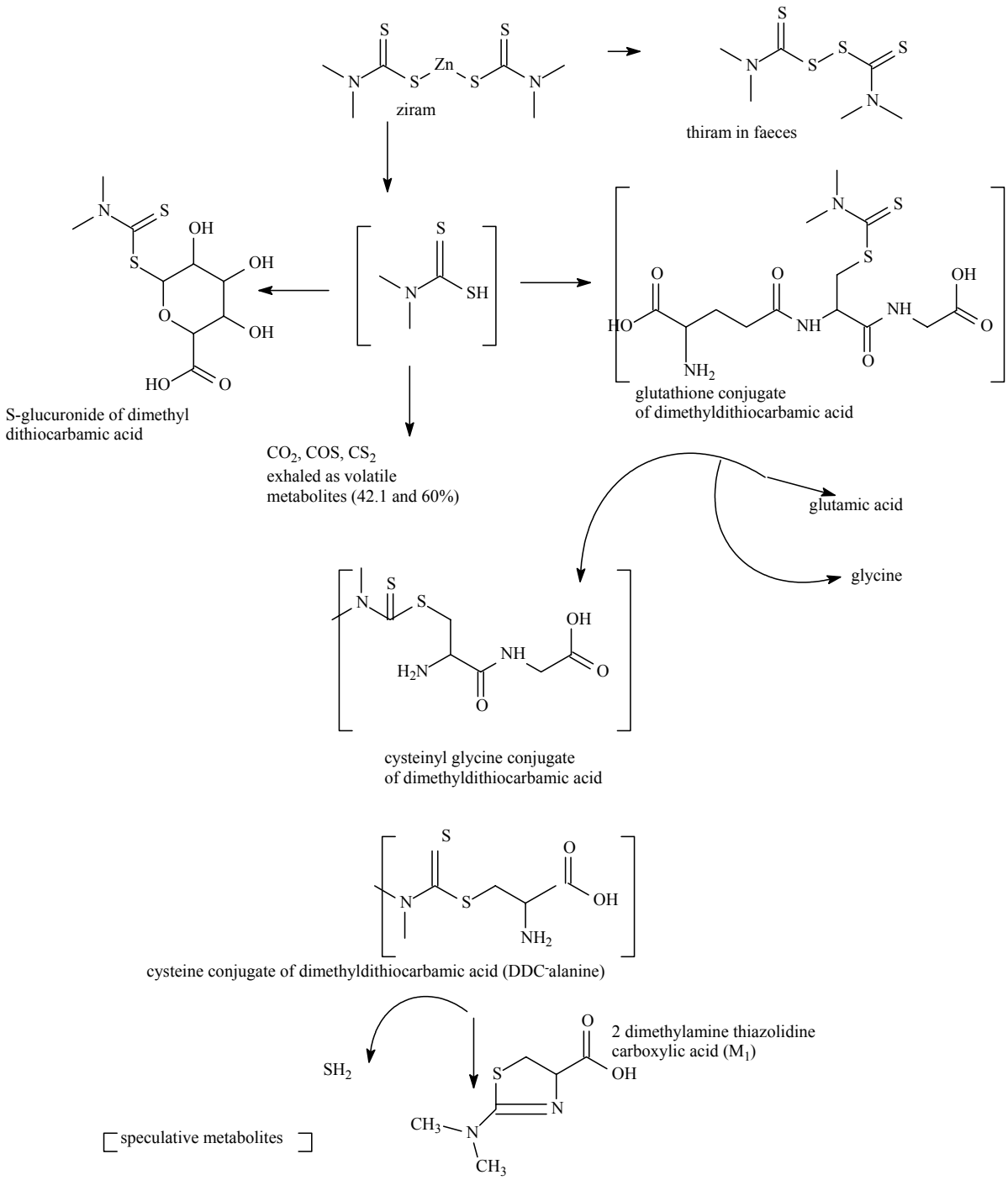
Conclusion:
In the monograph, it was concluded that the oral absorbed dose reached 58-61% after low dose and a correction factor of 50% was applied. Therefore, based on the new data, no further correction is necessary.

Distribution is rapid and large. High levels of radioactivity are detected in carcass, organs of metabolism and excretion as well as in high vascularised tissues. In the monograph, it was suggested that the compound did not accumulate, however, based on the kinetic parameters from the new study, we can expect that ziram may accumulate.

Following oral administration, radioactivity is essentially excreted as volatiles (CS_2, CO_2) in expired air (36-50%) within 24 h, reaching 42 to 60% of the dose within 168 h. Excretion via urine and faeces reaches 16% and 3% respectively. Overall excretion reaches 64 to 85% of the dose within 168 h.

Metabolism of absorbed dose is important. The principal route is hydrolysis to dimethyldithiocarbamate, which further decomposed to CS_2, COS and CO_2 exhaled as volatile metabolites or conjugated. Thiram is detected in faeces. In urine, S-glucuronide of dimethyldithiocarbamic acid and the 2-dimethylamine-thiazolidine carboxylic acid (M1) are detected.

Fig.B.6.1-1 proposed metabolic pathway of ziram



B.6.3.3.1 28 day inhalation toxicity in the rat (Annex IIA 5.3.3)

Due to the high inhalation toxicity of ziram, a 28-day inhalation toxicity was requested during the ECCO meeting.

Range finding study in rats: 5 day repeated dose snout only inhalation toxicity, 0.002, 0.005 and 0.015 mg/l (Brooker, 2000, Task force study)

Findings:
At the top dose, animals were sacrificed day 4 after exposure due to proliferative changes observed in larynx.
Body weight gain was decreased at all tested doses.
Histopathology of larynx: changes were seen at all dose levels. In the high dose group, the epithelial changes were generally both ulcerative/inflammatory and proliferative (hyperplasia/squamous metaplasia) in character, whereas in the low and intermediate dose groups the changes were predominantly proliferative (hyperplasia/squamous metaplasia).
In lungs, lesions were detected in all treated groups and demonstrated a dose-relationship. In the airways, epithelial hyperplasia was detected in the bronchioles and terminal bronchioles with prominent goblet cells seen in the major airways. In the alveolar ducts, granulomatous inflammation with increased mural fibrous tissue was detected. In addition, extravasation of eosinophils was detected.
Nasal turbinate: changes were variable, generally more common in females and confined to the high dose group. The lesions were detected at a low incidence in olfactory, respiratory and transitional epithelium and involved both degeneration and proliferative responses (Table B.6.3.3.1-1).

Table B.6.3.3.1-1: range-finding study: 28-day toxicity study in rats.

Exposure level	**0**		**0.002 mg/l = 0.540 mg/kg bw/d**		**0.005 mg/l= 1.35 mg/kg bw/d**		**0.015 mg/l = 4.05 mg/kg bw/d**	
	M	F	M	F	M	F	M	F
Body weight							↓ 12%	↓ 11%
Body weight **gain**			↓ 10%	↓29%	↓36%	↓74%	Killed day 4	Killed day 4
Food consump.			↓ 8 %	↑5%	↓ 6%	↑2%		
Water cons.			↓ 2 %	↑52%	↑13%	↑21%		
Organ weight:								
Lung, bronchi							↑28%	↑33%
Spleen							↓ 17 %	↓ 25%
Histopathological findings: 5 rats/sex examined								
Larynx								
Ventrolateral-epithelial ulceration	0	0	0	0	0	0	2	1
Ventrolateral squamous metaplasia	0	0	5*	5*	5*	4**	4**	4**
Ventral-epithelial hyperplasia	0	0	5*	5*	5*	5*	4**	3
Arytenoids-epithelial hyperplasia	0	0	2	3	3	2	5**	3
Ventral-epithelial ulceration	0	0	0	0	0	0	3	1
Lungs:								
Alveolar ducts-granulomatous inflamm. /fibrous tissue:								
	0	0	1	1	4*	3	5**	4*
Terminal bronchioles-epithelial hyperplasia:								
	0	0	0	0	4*	2	5**	3
Bronchiolar epithelium-prominent goblet cells:								

	0	0	4*	1	3	4*	5**	2
Extravasation of eosinophils:								
	0	0	3	2	5**	4*	4*	3
Bronchiolar epithelium hyperplasia:								
	0	0	0	0	0	0	5**	2
Nasal turbinate:								
Olfactory epithelium atrophy	1	0	0	1	2	0	2	4**
Tracheal and tracheal bifurcation:								
Epithelial hyperplasia	0	0	1	0	1	0	4**	4**
Epithelial hyperplasia bifurcation	0	0	3	0	3	3	5**	5**

* P<0.05; ** p < 0.01 with Fisher 's exact test

Conclusion: evidence of inflammatory, degenerative and proliferative changes was detected throughout the respiratory tract at all dose levels.

Guidelines: Experimental protocol in compliance with the test method B.8 of dir.96/54/EEC.
GLP status: yes (no attest of competent authority)
Material and methods:
Preliminary study: 5 rats/sex/dose were exposed by snout to ziram (batch n° V755/G8803961: 99.4 %) for 5 days, 6 hour/day at 0, 0.002, 0.005 and 0.015 mg/l. Animals were sacrificed 2 days after last exposure.
Conversion of mg/l in mg/kg bw/d: dose (mg/l) x 270.

Main study: 28-day repeat dose snout only inhalation toxicity study in rats at 0.000095, 0.000318, 0.001154 and 0.002898 mg/L with a 28 day reversibility period (Brooker, 2000, Task force study)

Findings:
Mortality: There were no unscheduled deaths during the course of the study.
There were no *clinical signs* that were treatment related.
Body weight gains for high dose males were statistically significantly higher than concurrent controls during the 4-week recovery period and this change was minor and not considered to be of toxicological importance.
Food consumption was not altered.
Haematology and clinical chemistry parameters showed occasional differences but in the absence of any correlation across the sexes and the absence of effects on any other parameters, were all considered to be of no toxicological importance.
There was a slight increase in lung weight of high dose male (a) and female rats (a +r), and high intermediate female rats (a) at the end of the 4-week exposure period. This finding was reversible following the 4-week period recovery (Table B.6.3.3.1-2).

Histopathological findings: as reported in Table B.6.3.3.1-3 and Table B.6.3.3.1-4, findings were observed in the larynx (squamous metaplasia of ventrolateral epithelium, hyperplasia of ventral epithelium, ventral pouch and arytenoids cartilage, necrosis of cartilage and inflammatory infiltration), lungs (fibrosis, inflammation, metaplasia in alveolar ducts, prominent goblet cells, mucous debris in airways, epithelial hyperplasia in bronchioles, bronchiolitis, increased incidence of foamy alveolar macrophages and extravasation of eosinophils), and tracheal bifurcation (epithelial hyperplasia).These effects were not observed at the lowest dose.
Following the 28-day reversibility period, there was complete recovery of the tracheal lesions, partial recovery of the lung lesions and some evidence of recovery of the laryngeal lesions (Table B.6.3.3.1-4).

Table B.6.3.3.1-2: main study: 28 day toxicity study in rats + 4 week recovery:

End Points	**0**		**0.1 µg/L= 0.027 mg/kg bw/d**		**0.3 µg/L= 0.081 mg/kg bw/d**		**1 µg/L= 0.27 mg/kg bw/d**		**2 µg/L = 0.81 mg/kg bw/d**	
	M	F	M	F	M	F	M	F	M	F
BW (g)										
4 wk	431	238	411	237	416	250	420	260	435	233
8 wk	524	267	-	-	-	-	-	-	568	258
Food consumption										
4 w k	218	136	202	139	207	143	202	146	206	135
8 wk	228	171							259	155
Haematology: 4 week										
MCHC							↓1%		↓1%	
leucocytes								↑200%		↑200%
Clinical chemistry: 4 week										
Globulin										↑16%
Glucose							↑23%		↑17%	
Organ weight –relative: week 5										
Lung+bronchi									↑14 %	↑11 %

Table B.6.3.3.1-3: 28-day toxicity study in rats - Microscopic pathology at week 5.

Endpoints	**0**		**0.095 µg/L**		**0.318**		**1.154**		**2.898**	
	M	F	M	F	M	F	M	F	M	F
Larynx: ventrolateral **squamous metaplasia**										
Minimal	0	0	0	0	3	0	0	0	0	0
Slight	0	0	0	0	1	1	1	0	0	0
Moderate	0	0	0	0	1	2	2	3	3	2
Marked	0	0	0	0	0	0	2	2	2	3
Total	0	0	0	0	5**	3	5**	5**	5**	5**
Ventral epithelial **hyperplasia**										
Slight	0	0	0	0	0	5	1	0	0	0
Moderate	0	0	0	0	0	0	4	5	5	2
Marked	0	0	0	0	0	0	0	0	0	3
Total	0	0	0	0	3	5**	5**	5**	5**	5**
Ventral **cartilage-necrosis**										
Slight	0	0	0	0	3	1	0	0	0	0
Moderate	0	0	0	0	0	3	1	1	1	1
Marked	0	0	0	0	0	1	4	4	4	4
Total	0	0	0	0	3	5**	5**	5**	5**	5**
Subepithelial inflammatory **infiltration**										
Slight	0	0	0	0	0	2	1	0	1	1
Moderate	0	0	0	0	0	0	4	5	4	4
Total	0	0	0	0	0	2	5**	5**	5**	5**
Liver: parenchymal **inflammatory cells**										
Slight	0	0	0	0	0	0	0	0	1	1
Clear cell foci										
Minimal	0	0	0	0	0	0	0	0	1	0
Lungs & bronchi: alveolar ducts										
Granulo.inflammation Slight	0	0	0	0	0	0	0	1	2	2

Fibrosis slight	0	0	0	0	0	0	2	1	1	2
Moderate	0	0	0	0	0	0	0	0	4	1
Total	0	0	0	0	0	0	2	1	5**	3
Bronchiolar metaplasia slight	0	0	0	0	0	0	0	0	4*	2
Terminal bronchioles-epithelial hyperplasia										
Slight	0	0	0	0	0	0	0	1	5**	3
Bronchiolitis	0	0	0	0	0	0	0	1	4*	1
Bronchiolar –epithelium- prominent goblet cells										
Slight	0	0	0	0	0	0	0	2	1	0
Moderate	0	0	0	0	0	0	1	0	2	1
Marked	0	0	0	0	0	0	0	0	1	0
Total	0	0	0	0	0	0	1	2	4*	1
Bronchiolar –epithelium- hyperplasia										
Slight	0	0	0	0	0	0	0	0	3	1
Lung & bronchi: Extravasation of eosinophils										
Slight/moderate	0	0	0	0	0	0	0	1	3 /1	2
Lung & bronchi mucous cellular debris in airways										
Slight	0	0	0	0	0	0	0	0	2	1
Moderate	0	0	0	0	0	0	0	0	1	0
Tracheal bifurcation: epithelial hyperplasia										
Slight	0	0	0	0	0	0	1	2	3	4
Moderate	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	0	0	0	1	2	3	5**

* P < 0.05; ** p< 0.01 with Fishers exact test

Table B.6.3.3.1-4: 28-day toxicity study in rats after 4-week recovery - Microscopic pathology.

Endpoints	**0**		**2.898**	
	M	F	M	F
Larynx:				
Ventrolateral **squamous metaplasia**				
Slight	0	0	3	2
Ventral epithelial **hyperplasia**				
Slight	0	0	3	0
Ventral **cartilage-necrosis**				
Moderate	0	0	1	0
Marked	0	0	4	5
Subepithelial **inflammatory infiltration**				
Slight	0	0	1	0
Lungs & bronchi:				
Alveolar ducts Fibrosis minimal	0	0	4	3
Aggre.alveolar macrophages	0	0	1	1
Tracheal bifurcation				
Loss of cilia	1	1	2	2

Conclusion:
There was a discussion with the applicant about the significance of the effects reported in the larynx. As suggested by the applicant and based on data from open literature, (Burger et al (1989, Toxicol. Applied

Pharmacol. 101, 521-542) and Gopinath et al (Atlas of experimental toxicological pathology, Vol 13, 1987)) some microscopic changes frequently seen in inhalation studies in rodents may be adaptative in nature. According to Lewis et al (Toxicol. Pathol. 19, 352-357) the reason (s) for susceptibility of the rat larynx to undergo squamous metaplasia following inhalation of a wide variety of chemicals is uncertain, but probably involves several factors, not least of these, is the location of isolated islands of ciliated cells in the middle of squamous regions at the basis of epiglottis, aside more likely to be subjected to inhaled insults than other respiratory areas. Similar changes have also been induced by the inhalation of glycerol and ascorbic acid and addition of ethanol 10% to inert pharmaceutical vehicle. Experiments in hamster and mice have shown similar results. However, administration of pharmaceuticals, previously shown to induce changes in rats, usually fails to result in the development of lesions in primates.
Induced laryngeal lesions commonly involve degeneration of original epithelial cells with hyperplasia and squamous metaplasia of ciliated and cuboidal epithelium and hyperplasia of squamous epithelium. The absence of atypia and dysplasia, and the usual absence of such lesions in primates suggest that the squamous metaplasia is probably only a defence mechanism (adaptative) by which a susceptible epithelium is replaced by a more resistant type.
The development of metaplastic changes inevitably presents interpretative problems with the extrapolation of findings to man.

In this study, the NOAEL is 0.1 µg/L taking into account the histopathological findings reported in larynx. **NOAEL = 0.027 mg/kg bw/d.**
If the larynx effects are considered to be a species-specific adaptative change, resulting from irritation, the **NOAEL** will be = **0.081 mg/kg bw/d**.

Guidelines: study protocol in compliance with test method B2 dir 84/449/EEC.
GLP status: draft report
Material and methods: 5 rats/sex/dose (Crl: CD BR, Sprague-Dawley) were exposed by inhalation using a snout-only exposure system to ziram technical white powder particulate aerosol (99.4%, batch n°. V755/98803961), 6 hour/day, 5 day/week for 4 weeks. Recovery study animals were retained for a further 4-week untreated.
Concentration in air: 0, 0.1, 0.3, 1.0, and 3.0-µg/L airs.
Overall analysed concentration of ziram: 0, 0.095, 0.318, 1.154 and 2.898 µg/L
Particle size :

Dose (µg/L)	Particule size		
	MMAD(µm)	Standard deviation	% < 7 µm
0.1	1.9	2.76	90
0.3	1.8	2.87	90
1.0	2.0	2.87	88
3.0	1.9	2.78	90

B.6.4 Genotoxicity (Annex IIA 5.4)

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

B.6.4.1.2 Gene mutation test in mammalian cells (Annex IIA 5.4.1)

No gene mutation test in mammalian cells was provided when the draft monograph was prepared. The study was provided to the RMS in October-November 1999.

- Mammalian cell mutation assay with ziram technical: forward mutations (TK) in mouse lymphoma cells L5178Y (Ransome, 1999, Task Force study) .

Findings:
Cytotoxicity was determined in the preliminary experiment by measuring relative survival (Table B.6.4.1.2-1).
Mutation tests: 2 tests were performed: T1 with 3 hour treatment (Table B.6.4.1.2-2) and T2 with 24 h treatment (Table B.6.4.1.2-3).
- Without S9 mix, a statistically significant increase in mutant frequency, which was outside the historical control range, was observed at 2 µg/ml in test 1. In test 2, no statistically significant increases in mutant frequency were observed even at levels of extreme toxicity.

- With S9 mix, no statistically significant increases in mutant frequency, which were outside the historical control range, were observed in test 1. In test 2, a statistically significant increase, outside the historical control, was observed at 3 µg/ml, but the D0 relative survival (4%) at this level was below the acceptable limit of toxicity.

Conclusion: no biologically significant increase in mutant frequency was observed at any of the tested concentrations. Ziram can be considered as non-mutagenic with or without metabolic activation when tested at concentrations extending into toxic doses.

Table B.6.4.1.2-1: preliminary cytotoxicity test.

Conc. Ziram technical (µg/ml)	**% Relative survival**		
	W/o S9 mix		**With S9 mix**
	3 h	**24 h**	**3 h**
0	100	100	100
0.01		113	
0.05		7	
0.1		0	
0.25		0	
0.5		0	
1		0	
1.96	26	0	51
2.5		0	
3.91	2		3
7.82	0		0
15.63	0		0
31.25	0		0
62.50	0		0
93.75	0		0
125	0		0

Table B.6.4.1.2-2: Mutation tests Test 1:

Conc. Ziram technical (µg/ml)	**% Relative survival**	**Mean relative %cloning efficiency**	**Mean mutant frequency**	**% Relative survival**	**Mean relative %cloning efficiency**	**Mean mutant frequency**
	W/o S9 mix			**With S9 mix**		
	D0	**D2**		**D0**	**D2**	
0	100	100	0.000179	100	100	0.000217
0.005	74			100		
0.01	109			111		
0.05	106			91		
0.1	78	93	0.000203	84	88	0.000219
0.25	100	70	0.000259	103	76	0.000252
0.5	90	82	0.000301	106	84	0.000230
1	71	68	0.000299	118	86	0.000222
2.00	26	58	0.000377**	52	82	0.000302
3.00	0			0		
MC 2.5µg/ml				97	73	0.001327**
MMS 10µg/ml	47	56	0.001356**			

** Statistically significant increase outside historical control range

Table B.6.4.1.2-3: Mutation tests Test 2.

Conc. Ziram technical (µg/ml)	% Relative survival	Mean relative %cloning efficiency	Mean mutant frequency	% Relative survival	Mean relative %cloning efficiency	Mean mutant frequency
	w/o S9mix			With S9mix		
	D0	D2		D0	D2	
0	100	100	0.000153	100	100	0.000129
0.0001	50					
0.0005	109					
0.001	97	78	0.000173			
0.0025	57	86	0.000159			
0.005	71	70	0.000188			
0.01	58	69	0.000206			
0.025	4	58	0.000171			
0.05	0			81		
0.1	0			60		
0.25				80		
0.5				81		
1				83	82	0.000145
1.5				70	97	0.000119
2.00				40	80	0.000131
2.5				11	66	0.000316
3.00				4	57	0.000383
MC 2.5µg/ml				74	78	0.000601**
MMS 5µg/ml	60	57	0.001505 **			

** Statistically significant increase outside historical control range.

Guidelines:
Experimental protocol in compliance with test method B.17 of directive 96/54/EEC and with TG OECD 476 (1997).
GLP status: the study is GLP.
Material and methods:
Mouse lymphoma L5178Y/TK+/- cell suspensions were exposed to Ziram (UCB 99.4 %, lot n°. V755/G8803961) with or without S9 (rat liver treated with Aroclor 1254). A pretest and a main test were performed.
Preliminary toxicity test:
3 h treatment (+/- S9), doses: 1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 93.75, 125 µg/ml
24 h treatment (-S9), doses: 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 µg/ml
Mutation tests:
-S9: test 1 (3 h treat.): 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2, 3 µg/ml
Test 2 (24 h treat.): 0.0001, 0.0005, 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1 µg/ml
+ S9: test 1 (3-h treat.) : 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2, 3 µg/ml
Test 2 (3-h treat.) : 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 µg/ml
Positive control: methyl methanesulphonate (-S9), 3-methylcholanthrene with S9.Solvent was DMSO.

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

In the monograph, a 2-year rat study was provided from which no NOAEL could be derived.
A long-term rat study of ziram was published in the open literature by Enomoto et al., 1989 and Maita et al., 1997 and was summarised in the monograph.
The full report of the study was made available to the RMS on April 1999 by the applicant and was re-evaluated on this basis.

Twenty-four-month dose chronic toxicity and carcinogenicity study of ziram in rats (Harada et al., 1983)

Findings:
Main findings are described in table B.6.5.1-1. The MTD was reached.

Mortality: a slight increase in cumulative death rate in male top dose group was observed and was mainly due to foreign body pneumonia and rhinitis. No such an effect was observed in females. These effects were considered not treatment-related, since the same respiratory lesions were also observed in the control group.
Clinical signs: abnormal changes in the hind limbs were observed in 3 males of the top dose group at the terminal stage of the study. Lose of vigour in hind limbs from the knee joint to the crural part and paralytic-like signs were observed. These changes were considered to be due to tibia abnormalities observed in macroscopic, X-ray and histological examination.
Haematology: in the 2000-ppm group, slight but significant decrease in hematocrit, haemoglobin and RBC count were observed in males in week 78 and anaemic tendency. In females, significant decrease in hematocrit, haemoglobin and RBC count were observed in week 52 and 78 and mean corpuscular volume, mean corpuscular haemoglobin and reticulocyte count also increased. These changes recovered almost completely in week 104 where only decreased RBC counts and increased corpuscular volume were still observed.
Blood chemistry: at 2000 ppm, from week 52 for males, and week 26 for females, glucose, and total cholesterol were low, reflecting the low nutrition state of the animals. Significant decrease in calcium is probably related to the thyroid and bone pathology and strongly suspected as a compound-related effect.
Organ weight: decreases in absolute and relative weights of the crural muscle were observed in males at 200 ppm and 2000 ppm from week 52 and in females from week 78 of administration.
Spleen increased weight was considered to be a secondary change in response to anaemia observed in haematology. Increased liver weight was not associated with abnormal histology.
Necropsy findings: at the top dose, hypertrophy of thyroid and atrophy of the crural muscle were observed with a high frequency in males and females at killing schedule in week 52 and 104 of administration. In males of the same group, proximal arcuation of the tibia and extension deficiency of the knee joint were observed sporadically in some animals at the end of examination. In the soft X-ray examination of the diseased limbs of animals with severe changes, clear extension of the tibia, proximal arcuation of the knee joint, and dilatation of the epiphysis were observed. Such changes were not observed in animals given 200 ppm or less.
Histopathology of non-neoplastic lesions:
At 2000ppm:
Follicular hypertrophy of the thyroid was observed (sometimes at 200 ppm). This effect was considered to be related to treatment, but there were no proliferative changes of increase in frequency of tumours and the change was considered to be rather the regressive lesion.
In the stomach, increased mucus cornification of anterior stomach was increased from week 26 and was considered to be a local response to the direct stimulation in the gastric wall by ziram.
In bone marrow, haematogenesis was increased from week 79 in males and in females; extramedullary haematopoiesis of the spleen was increased in some periods.
In the liver, small granulomas were observed in males, but there were no noticeable enzymatic or histological changes.
In males, dilatation of the oesophageal cavity was related to onset of foreign body nasal catarrh and pneumonia.
In addition, increases in inner bronchial groove remnant of the thyroid, sinus dilatation of the adrenals, foreign body pneumonia and rhinitis, and dilatation of oesophageal cavity which were observed in the treated groups were considered to be probably due to incidental increase in background lesions and not directly related to ziram.
In the knee-joint, excessive remnant of chondrial plate of the epiphysis (delay in closure plate of epiphysis), which is usually closed with ageing, was observed in 22 of the 77 males and in 13 of the 73 females. The same lesions were observed mainly in the tibia of males and in both tibia and femur of females. Furthermore, chondrial plate of epiphysis of the tibia was hypertrophied in part of the males because of hyperplasia of cartilage cells and hyperplasia of the neo-bone trabecula. These changes were not due to direct action of ziram on the cartilage cells, but rather the decrease in calcium observed in the blood biochemical examination and the relationship to the above-mentioned thyroidal lesions suspected.
In the crural muscle, focal muscular atrophy was observed from week 52 of administration and in sciatic nerve, nerve fibre degeneration was observed in males and females with a high frequency in week 104 of administration. This lesion was clearly due to ziram, but since atrophy of the muscle fibre was localised, neurogenicity was suspected, and the relationship to nerve fibre degeneration of the sciatic nerve, which increased in males and females of 2000-ppm group was spotlighted.
At 200 ppm: total frequency of follicular hypertrophy of the thyroid in females and increased cornification of anterior stomach and atrophy of the crural muscle increased significantly from week 78.
At 20 ppm: there were no differences with the control (Table B. 6.5.1-2).

In neoplastic lesions, the kind and onset time and frequency of the lesions developed in each treated group were similar to those in the control group, and there were no marked differences between the results of the present study and those in the control group of the other toxicity studies performed in the laboratory (Table B. 5.5.1-3).

Table B. 6.5.1-1: 104-week toxicity study in rats.

Endpoints/dose	**0 ppm**		**20 ppm**		**200 ppm**		**2000 ppm**	
	M	F	M	F	M	F	M	F
Mortality After 12 month After 26month	 0/56 15/56	 0/56 21/56	 1/56 12/56	 2/56 17/56	 1/56 15/56	 4/56 23/55	 0/56 22/56	 2/56 22/56
Clinical signs			See text					
Body weight changes							↓20 %	↓ 27 %
Food consumption							(↓11%)	(↓13%)
Water consumption							↑17% week1↑80	↑9 % week14↑48
Urinalysis: Specific gravity					↓0.5 %		↓ 2 %week 52↑104	↓ 2 %week 26, 52
Haematology: MCV MCH RBC							↓ 3 %* ↓ 4%*	↑5%* ↓23%**
Clinical chemistry Glucose Cholesterol Ca^{2+} Bilirubin							↓ 12 %** ↓44 %*** ↓ 5 %***	↓7 %* ↓ 7%*** ↓ 37%*
Rel. organ weight Liver Spleen Testes Crural muscle Thyroid				↓4%*	↓25%***	↓22%***	↑15%*** ↑24%* ↑82%*** ↓27%***	↑19%*** ↓15%*** ↑78%***
Necropsy findings Thyroid swelling, hypertrophy Crural muscle atrophy	5/80 0/80	1/80 1/80	4/80 0/80	4/80 0/80	12/80 4/80	4/80 2/80	29/80 34/80	15/80 23/80

*, **; *** Statistically significantly different from control at 5, 1 or 0.1% level of probability.
(): Not statistically significant

Table B. 6.5.1-2: 104-week toxicity study in rats: non-neoplastic lesions.

Endpoints/dose	**0 ppm**		**20 ppm**		**200 ppm**		**2000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N°. Animal examined	80	80	80	80	80	80	80	80
Spleen: increased brown pigmentation	4	21	4	26	7	34	10	31

Congestion	3	6	6	2	4	7	10	6
extramedullary haematopoiesis		13		16		14		34***
Liver: small granuloma	5		10		10		15*	
Thymus: lymphocyte hyperplasia	1		2		2		5	2
Oesophagus cavity dilatation	3	14	3	8	5	22	21**	15
Stomach mucus cornification	5	1	1	6	13	11**	46	34**
Lung: foreign body pneumonia	3	12	2	8	4	16	17**	15
Pancreas fibrosis	5	2	5	1	1	2	10	8
Thyroid: acinous hypertrophy	2	1	4	1	3	9**	64***	66***
Inner bronchial groove remnant	1		3	1	4	4	17***	29***
Adrenal :								
sinus dilatation	3	7	4	4	6	10	13**	37***
Medullar hyperplasia	2		3		4		6	
Sciatic nerve:								
Nerve fibre degeneration			2	2			30***	26***
Bone marrow:								
Increased haematogenesis	10	7	10	4	4	3	21*	23**
Bone and knee joint:								
Delay in closure of chondrial plate of tibia epiphysis		1				5	22***	13***
Chondrial cell hyperplasia of tibia epiphysis							6	
Crural muscle atrophy	1	2	4		49***	25***	68***	46***
Nasal cavity rhinitis:	7	10	6	7	7	21*	37***	32***
Auditory canal otitis:	4	12	3	10	5	25	13	17

*, **, ***: Statistically significant at p<0.05, <0.01, <0.001: level of significance by Fisher's exact probability test.

Table B. 6.5.1-3: 104-week toxicity study in rats: neoplastic lesions.

End points/dose	0 ppm		20 ppm		200 ppm		2000 ppm	
	M	F	M	F	M	F	M	F
N°. Animal examined	80	80	80	80	80	80	80	80
Liver: nodular cell hyperplasic foci		2	1			1	3	3
Perputal adenoma	3		3		4		8	
Thyroid:								
C- cell adenoma	11	6	11	6	15	6	7	1
C- cell adenocarcinoma			1	1	3	2	1	1
Follicular adenoma	1						1	
Follicular adenocarcinoma			1				1	
Total benign tumours	105	51	102	46	106	33	100	23
Total malignant tumours	18	14	13	16	17	6	13	10
Total benign and malignant tumours	123	65	115	62	123	39	113	33

Conclusion: ziram is not carcinogenic in this study.
NOAEL = 20 ppm = 0.56 mg/kg bw/d.

Guidelines: Experimental protocol not fully in compliance with Dir. 87/302/EEC annex V. B or OCDE 453 (1981).
Deviation from official protocol: haematology not performed at 3 month. Urinary volume not measured and sediment not examined.
GLP status: no attest of study director, attest of QAU, no attest of competent authority.
Material and methods:
80 Fischer rats (F344/DuCrj strain, SPF)/sex/dose received ziram (97.5%) in the diet at 0, 20, 200 or 2000 ppm for 24 months. 8 animals /sex/dose were sacrificed at week 26, 52 or 78.
Stability of ziram in diet: The dietary admixture was prepared 3 times a week. The results of stability of ziram in diet indicate that integrity of ziram in test diets could be preserved 80% or more in the 20 and 200-ppm diet. At 2000 ppm 90% of ziram was preserved.
Taking the aspect of instability of ziram in diet into account, at doses of 20 to 200 ppm, it is necessary to apply a correction factor of 20% and of 10% for the 2000-ppm estimated dietary intake.
Converted dose intake: for male: 0.56; 5.52 or 66.6 mg/kg bw/d
For females :0. 664; 6.8; 81.9 mg/kg bw/d
The study is accepted.

B.6.10.2 Acceptable Daily Intake (ADI) (Annex IIA5.10)

An ADI is proposed on the basis of the long-term toxicity study in rats in which a NOAEL of 0.56-mg/kg bw/d is acceptable. An assessment factor of 100 is proposed.

ADI = 0.006 mg/kg bw/d

B.6.10.4 Acceptable Operator Exposure Level (AOEL) (Annex IIA5.10)

In the monograph, a systemic AOEL short-term was calculated on the basis of a NOAEL from the 90-day rat study, satellite study of 104-week rat study, of 3.2-mg/kg bw/d. A correction factor of 50% was applied for oral absorption and an assessment factor of 100 was used for extrapolation to human, giving a

AOEL systemic = 0.016 mg/kg bw/d.

In the monograph, a NOAEL for dermal exposure was also proposed which was based on a 21 dermal rabbit study.

AOELdermal = 1 mg/kg bw/d.

During the ECCO 78, it was concluded that a repeat-dose inhalation study was necessary.
A NOAEL can de derived from the lowest tested dose of 0.027-mg/kg bw/d, based on the histopathological findings reported in the larynx at the next higher dose of 0.081-mg/kg bw/d. However, if these effects are a species-specific irritating effect, the higher dose of 0.081-mg/kg bw/d can be used for setting of AOEL.

Applying an assessment factor of 100 will give an

AOELinhalation = 0.00027 mg/kg bw/d.
Or
AOEL inhalation = 0.00081 mg/kg bw/d

B.6.14 Exposure data (Annex IIIA 7.2)

B.6.14.1 Estimation of operator exposure (annex IIIA 7.2.1.1)

In the monograph, Ziram WG formulations were used in orchards (pomefruit, stone fruit, and almond). The application rates are in the range of 1.18 to 3.04 kg a.s. /ha. Spray concentrations are in the range 75-304-532 g a.s. /hl. At maximum, 4 to 6 applications per season can be applied with an interval of 7-14 days. Main application occurs through atomisers or sprayers between 1000 and 2000-l water per ha but can change depending on, the area, and the type of material used and the size of the trees. The applicant proposes to reduce the application rate to 2.28 kg/ha in place of 3.04 kg a.s. /ha as well as the number of application to a maximum of 4 during the season with a spray interval of 7-14 days. Ziram must be thoroughly sprayed all over the foliage to ensure a good protection. Therefore, large volumes of water are being used: from 1000 litres per hectare for pear, apple, to 1500 litres for cherry, plum, depending on the size of the three.

Table B.6.14.1-1: Orchard treatment (cherry, plum, peach), 2.28 kg/ha, 228 g a.s. /hl.

	UK POEM model	German model
	Use rate: 2.28 kg a.s. /ha	
Number ha treated/day	30 ha/day	8 ha/day
Method of use:	Vehicle air assisted broadcast > 400 l/ha	High crop tractor mounted
AOEL dermal	= 1 mg/kg bw/d	
AOELinhalation	= 0.00027 mg/kg bw/d (Based on the NOAEL= 0.027 mg/kg bw/d; AF= 100)	
AOEL systemic	= 0.016 mg/kg bw/d	
Body weight	60 kg	70 kg
Dermal absorption:	5% during M/L 30% during A	

- **Operator exposure according to the German and UK POEM models:**

In a first approach, exposure estimation was made using the UK POEM and the German model using the reduced application rate of 2.28 kg/ha.
As shown in table B.6.14.1-2, when no protections are used, operator exposure is above the AOELdermal and AOELinhalation. Operator exposure is then estimated using protective equipment such as gloves in the UK model and gloves as well as inhalation protection in the German model. The operator exposure was higher than the AOELs when considering the UK model. The operator exposure is acceptable according to the German model when protective equipment (gloves, inhalation, half mask) is applied.

- **Measurement of operator exposure:**

In the monograph, a study was reported in which measurement of operator exposure to ziram was described. The data reported in the study were corrected for the reduced application rate (2.28 kg/ha) and a route-to-route extrapolation is proposed (table B.6.14.1-2).
In these conditions, operator exposure is acceptable without PPE (0.0122 mg/kg bw/d, 76 % of AOELsyst) for a maximal treatable area of 8 ha, 2.28 kg/ha, which is also a worst case for the German model.
The evaluation of operator exposure was also realised considering protective equipment. When gloves are recommended during mixing/loading and application as well as a half mask (disposable half-mask EN 143:FFP2 SL) during preparation of the spray and application, operator exposure is below the systemic AOEL (0.00295 mg/kg bw/d = 18 % of AOELsyst) and the route to route extrapolation shows that exposure is also acceptable (0.00273 mg/kg bw/d = 0.27 % of AOELdermal and 0.000228 mg/kg bw/d = 84 % of AOELinhalation).

Table B.6.14.1-2: Operator exposure as calculated according to the UK POEM and the German model and comparison of data from measurement of operator exposure with AOELdermal and AOELinhalation.

	UK model	**German model**
	2.28 kg/ha, 30 ha/day UK conditions	**2.28 kg/ha, 8 ha/day German conditions**
Absorbed dose (mg/kg bw/d) and % of respective AOEL		
No protection:		
Total	1.147 mg/kg bw/d = 7168 % AOELsyst	0.707 mg/kg bw/d = 4418 % AOELsyst
Dermal	1.138 mg/kg bw/d =113 % of AOEL	0.702 mg/kg bw/d = 70.2% of AOEL
Inhalation	0.0085 mg/kg bw/d = 3148% of AOEL	0.005 mg/kg bw/d = 1851% of AOEL
With gloves (UK and German model) + RPE-FF2-SL-P2 for inhalation during M/L (German model)		
Total	0.747 mg/kg bw/d = 4668 % of AOELsyst	0.576 mg/kg bw/d =3604% AOELsyst
Dermal	0.739 mg/kg bw/d= 74% of AOEL dermal	0.570 mg/kg bw/d = 57% of AOEL
Inhalation	0.0085 mg/kg bw/d = 3148% of AOEL	0.00364 mg/kg bw/d = 1348% of AOEL
With gloves (UK and German model) + RPE-FF2-SL-P2 for inhalation during M/L + application (German model)		
Total		0.570 mg/kg bw/d = 3566 % AOELsyst
Dermal		0.570 mg/kg bw/d = 57% of AOEL
Inhalation		**0.000257 mg/kg bw/d = 92% of AOEL**
Measurement of exposure: performed w/o cabs or open cabs		
No PPE	2.28 kg/ha, 30 ha/day UK conditions	2.28 kg/ha, 8 ha/day German conditions
Absorbed dose (mg/kg bw/d) and % of respective AOEL		
Total	0.0764 mg/kg bw/d = 477 % of AOEL syst	**0.0122 mg/kg bw/d = 76 % of AOELsyst**
Dermal route	0.059 mg/kg bw/d = 5.9% of AOEL	0.00767 mg/kg bw/d = 0.76 % of AOEL
Inhalation	0.0174 mg/kg bw/d = 3166% of AOEL	0.00456 mg/kg bw/d = 1688% of AOEL
+ PPE		
Total	0.0557mg/kg bw/d = 348% of AOEL syst	**0.00295 mg/kg bw/d = 18 % of AOELsyst**
Gloved hands	0.0383 mg/kg bw/d=3.8% of AOEL	0.00273 mg/kg bw/d = 0.27 % of AOEL
+ Half mask during ML/A	-	**0.000228 mg/kg bw/d = 84 % of AOELinhalation**

(% Of AOEL inhalation = 0.00081 mg/kg bw/d)

Conclusion: using PPE makes operator exposure to ziram acceptable for operator as operator exposure is below the AOELsystemic, AOELdermal and AOELinhalation.
This risk assessment is a real worst case scenario as irritating effects observed in the 28 day inhalation rat study were taken into account, knowing that they are probably not relevant for human.

Use of a half-mask during application
Comments of the notifier:
The type of half -mask recommended by the notifier is an anti-aerosol (liquid and solid) disposable half mask (filtration of 95% of the particles with a diameter > 0.6µm), which should not be considered as a big constraint under practical conditions (easy to use, disposable, cheap)

Comments about the use of the subacute inhalation toxicity rat study and route-to-route extrapolation:
The 28-day inhalation study on rats has been conducted with ziram technical aerosol powder and very fine particles were used in the study (90% with a MMAD <7µm= a respirable aerosol), which are not considered relevant to those generated during application of ziram by airblast sprayers.
In practice, during application, farmers are exposed during /mixing loading to granules, and to spray droplets during application (ziram suspended in water droplets), the formulation being a WG. (According to the guidelines, acute inhalation toxicity studies should not be performed when a formulation is a WG). The nozzles mounted on the sprayers used in the orchards produce 90% of droplets with a size >100 µm and, as reported in the guidance document on "particle size distribution, fibre length and diameter distribution (ECB/TM/February 1996), particle with an aerodynamic diameter of >100µm are not included in the inhalable convention and do not contribute to inhalation toxicity.

RMS considers that a route-to-route extrapolation is in this case not realistic.

B.6.14.3.a estimation of bystander exposure.

Considering a spray drift of 4.5% (distance of 10 m between a bystander and the treated orchard), the dermal and inhalation exposures of bystander (without PPE) can be estimated as follows:
The measurement of inhalation exposure given in the operator exposure study (Meikle, 1991) is 0.05 µg/kg bw/kg a.s. (during application).
Thus, the absorbed dose by inhalation for an application of 2.28 kg/ha and 8 ha is:
$E_{inhalation}$ = (0.05 x 0.045 x 2.28 x 8) = 0.041 µg/kg bw /d < $AOEL_{inhalation.}$

The dermal absorbed dose during application given in the operator exposure study is:
E_{dermal} = 0.945 µg/kg bw/kg a.s. x 0.045 x 2.28 x 8 = 0.77 µg/kg bwday < $AOEL_{DERMAL}$

Therefore, taking into consideration the above calculations, the risk for the bystanders during application of ziram should be low.

- Spontaneous tumours in F344/DuCrj rats from 12 control groups of chronic and oncogenicity studies from the same laboratory as which one performed the above mentioned carcinogenicity study in rat (Maita et al., 1987).

Results from studies carried out during a 1978-1983 period in 960 males and 959 female rats used as controls in 12 chronic and oncogenicity studies.

Findings:
C-cell adenoma of the thyroid usually occurred unilaterally but was not rare to arise bilaterally. The criteria to define between hyperplasia and adenoma are much debatable. Maita Criterion for C-cell adenoma was based on size of the lesion, and more than one follicular size was diagnosed as adenoma. When the tumour cells showed a proliferation beyond the capsule, or metastasis to the neighbouring lymph node or lung, adenocarcinoma was given as a diagnosis. C-cell adenoma was seen predominantly in males. These results suggest that thyroid tumours have a high rate of spontaneous occurrence.

1. Age-related occurrence of tumours in **male F344/DuCrj rats** at more than 5% incidence and variability in incidence among 12 control groups:

Histological types of tumours	**Overall incidence of tumours and variability in incidence**	**Age-related occurrence of tumours N° affected (%)**			
		0-58 wk.	**59-84**	**85-97**	**98-110**
N°. Of animals examined during each interval of age	960	198	144	57	591
Leukaemia (Mononuclear cell)	7.1 (3.8-12.5)	3 (1.5%)	6 (5.3)	12 (21.1)	47 (8)
Testis: interstitial cell tumour	74.6 (63.8-82.5)	4 (2)	88 (77.2)	41(71.9)	583(98.6)
Preputial gland: adenoma	5.3 (1.3-12.5)	1 (0.5)	3 (2.6)	4 (7)	43 (7.3)
Pituitary: anterior adenoma	12.4 (7.5-18.8)	-	6 (5.3)	7 (12.3)	106 (17.9)
Thyroid: C-cell adenoma	**12.5 (3.8-21.3)***	**-**	**6 (5.3)**	**5 (8.8)**	**109 (18.4)**
Adrenal: pheochromocytoma	11.9 (1.3-22.5)*	3(3.8)	6 (5.3)	9 (15.8)	96 (16.3)
Subcutis : fibroma	7.2 (5.0-11.3)	-	2 (1.8)	6 (10.5)	61(10.3)

* Significant heterogeneity p<0.05 Fisher's exact test.

2. Age-related occurrence of tumours in **female F344/DuCrj rats** at more than 5% incidence and variability in incidence among 12 control groups:

Histological types of tumours	**Overall incidence of tumours and variability in incidence**	**Age-related occurrence of tumours N° affected (%)**			
		0-58 wk.	**59-84**	**85-97**	**98-110**
N°. Of animals examined during each interval of age	959	204	141	45	568
Leukaemia (Mononuclear cell)	9.5 (3.8-15)	- (-)	4 (2.8)	9 (20)	78 (13.7)
Uterus: endometrial polyp	12.6 (6.3-23.8)	3 (1.5)	9 (6.3)	3(6.7)	106(18.7)
Preputial gland: adenoma	5.3 (1.3-12.5)	1 (0.5)	3 (2.6)	4 (7)	43 (7.3)
Pituitary: anterior adenoma	28.2 (15-41.3)*	-	25 (17.6)	9 (20)	236 (41.5)
Thyroid: C-cell adenoma	**8.2 (2.5-16.3)**	**1 (1.3)**	**4 (2.8)**	**2 (4.4)**	**72 (12.7)**

Mammary gland: adenoma/fibroadenoama	13.2 (5-22.5)	-	3 (2.1)	2 (4.4)	122 (21.5)

* Significant heterogeneity p<0.05

Operator exposure:

UK POEM:
Tractor drawn air assisted orchard sprayer 500-l/ha models

Product data	
Product	Ziram 760 WG
Active substance	Ziram
Concentration	760 mg/g
Formulation type	WDG
Maximum in use a.s.concentration	1.7328 mg/ml

Exposure during mixing and loading	
Container size	5 kg
Hand contamination/operation	0.01 ml
Application rate	2.28 kg product/ha
Work rate	30 ha/day
Number of operations	14 / day
Hand contamination	0.14 g/day
Protective clothing	None
Transmission to the skin	100 %
Dermal exposure to formulation	0.14 g/day

Exposure during spray application
Application technique- Tractor drawn air assisted orchard sprayer with hydraulic nozzles

Application volume	1000 l 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	0.14 g/day	121.2 ml/day
Concentration of a.s.	760 mg/ml	1.7328 mg/ml
Dermal exposure to a.s.	106.4 mg/day	210.01536 mg/day
Percent absorbed	5%	30%
Absorbed dose	5.32 mg/day	63.004608 mg/day

Inhalation exposure during spraying	
Inhalation exposure	0.05 ml/h
Duration of exposure	6 h
Concentration of a.s.	1.7328 mg/ml
Inhalation exposure to a.s.	0.51984 mg/day
Percent absorbed	100%
Absorbed dose	0.51984 mg/day

Predicted exposure	
Total absorbed dose	68.844448 mg/day
Operator body weight	60 kg
Operator exposure	1.147407467 mg/kg bw/d

Tractor drawn air assisted orchard sprayer 500-l/ha model

Product data			
Product	Ziram 760 WG		
Active substance	Ziram		
Concentration	760 mg/g		
Formulation type	WDG		
Maximum in use a.s.concentration	1.7328 mg/ml		
Exposure during mixing and loading			
Container size	5 kg		
Hand contamination/operation	0.01 ml		
Application rate	2.28 kg product/ha		
Work rate	30 ha/day		
Number of operations	14 / day		
Hand contamination	0.14 g/day		
Protective clothing	Gloves		
Transmission to the skin	100 %		
Dermal exposure to formulation	0.0014 g/day		
Exposure during spray application			
Application technique- Tractor drawn air assisted orchard sprayer with hydraulic nozzles			
Application volume	1000 spray/ha		
Volume of surface contamination	400 ml/h		
Distribution	Hands	Trunk	Legs
	10	65	25
Clothing	None	Permeable	Permeable
Penetration	10	2	5%
Dermal exposure	4	5.2	5 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray			
Absorbed dose	Mix/load	Application	
Dermal exposure	0.0014 g/day	85.2 ml/day	
Concentration of a.s.	760 mg/ml	1.7328 mg/ml	
Dermal exposure to a.s.	1.064 mg/day	147.63 mg/day	
Percent absorbed	5%	30%	
Absorbed dose	0.0532 mg/day	44.29 mg/day	
Inhalation exposure during spraying			
Inhalation exposure	0.05 ml/h		
Duration of exposure	6 h		
Concentration of a.s.	1.7328 mg/ml		
Inhalation exposure to a.s.	0.51984 mg/day		
Percent absorbed	100%		
Absorbed dose	0.51984 mg/day		
Predicted exposure			
Total absorbed dose	44.863 mg/day		
Operator body weight	60 kg		
Operator exposure	0.747723 mg/kg bw/d		

GERMAN MODEL

Use information

Product	ziram	Active substance	Ziram

Formulation type	WG	A.s. concentration	760 mg/g
Method of use	tractor high crops	Dose (product)	2.28 kg product/ha
Work rate	8 ha/day	Dose (a.s.)	1.732 kg a.s./ha
		Amount handled	13.86 kg a.s./day

1. Without PPE:
Exposure-mix/loading

	PPE (including RPE)	Estimated exposure (PPE)	
Inhalation	None	0.110848 mg a.s./day	
Dermal-hands	None	27.712 mg a.s./day	
Exposure application			
Inhalation	None	0.2494 mg a.s./day	
Dermal -head	None	16.6272 mg a.s./day	
Dermal -hands	None	9.6992 mg a.s./day	
Dermal- body	None	133.0176 mg a.s./day	
Total exposures			
	Percent absorbed	Estimated exposure mg a.s./day	Percent absorbed mg/kg bw/day
Total potential inhalation	100%	0.360256	0.005
% of AOEL inhal.			**1851%**
Total Dermal-mix	5%	27.712	0.0197
Total dermal-application	30%	159.344	0.6829
% of AOEL dermal			**0.702 mg/kg bw/d = 70%**
Total absorbed dose			0.707 mg/kg bw/day
% of systemic AOEL			**4418%**

2. With PPE : gloves (M/L + A)+ half mask during mixing/loading
Exposure-mix/loading

	PPE (including RPE)	Estimated exposure (PPE)	
Inhalation	RPE-FF2-SL or P2	0.00554 mg a.s./day	
Dermal-hands	gloves	0.27712 mg a.s./day	
Exposure application			
Inhalation	None	0.2494 mg a.s./day	
Dermal -head	None	16.6272 mg a.s./day	
Dermal -hands	gloves	0.0 9699 mg a.s./day	
Dermal- body	None	133.0176 mg a.s./day	
Total exposures			
	Percent absorbed	Estimated exposure mg a.s./day	Percent absorbed mg/kg bw/day
Total potential inhalation	100%	0.2549	0.00364
% of AOEL inhal.			**1348%**
Total Dermal-mix	5%	27.712	0000197
Total dermal-application	30%	159.344	0.570
% of AOEL dermal			**57%**
Total absorbed dose			0.573
% of systemic AOEL			**3604%**

2. With PPE : gloves (M/L + A) + half mask during ML + A
Exposure-mix/loading

	PPE (including RPE)	Estimated exposure (PPE)

Inhalation	RPE-FF2-SL or P2	0.00554 mg a.s./day	
Dermal-hands	Gloves	0.27712 mg a.s./day	
Exposure application			
Inhalation	RPE-FF2-SL or P2	0.01247 mg a.s./day	
Dermal -head	None	16.6272 mg a.s./day	
Dermal -hands	Gloves	0.0 9699 mg a.s./day	
Dermal- body	None	133.0176 mg a.s./day	
Total exposures			
	Percent absorbed	Estimated exposure mg a.s./day	Percent absorbed mg/kg bw/day
Total potential inhalation	100%	0.0180	0.000257
% of AOEL inhal.			**92%**
Total Dermal-mix	5%	27.712	0000197
Total dermal-application	30%	159.344	0.570
% of AOEL dermal			**57%**
Total absorbed dose			0.570
% of systemic AOEL			**3566%**