ANNEX B

Ziram

B.5 Toxicology and metabolism

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

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CH3            S            S         CH3
    \         //            \\       /
     N  -  *C - S - Zn - S - * C  -  N
    /                                 \
CH3                                    CH3
```

***** position of ^{14}C labeling

Zinc dimethyl dithiocarbamate belongs to the dialkyldithiocarbamates.

Material and methods :

-Metabolism of Ziram in rats (Cheng, 1989) (Ziram task force)
Guidelines :
Experimental protocol of the study partially in compliance with test method B. directive 87/302/EEC.
Deviation from the official protocol: Distribution was not investigated at different times after exposure.
GLP status: yes
Material and methods :
5 Male and female rats/dose (Crl:CD® (SD)BR) received a single oral dose of Ziram (specific activity: 13.9 mCi/mmol; radiochemical purity : 96.9%) at 15 or 352 mg/kg bw or 15 daily oral doses of 15 mg/kg bw (a single radiolabeled dose on day 15) in 0.5% carboxymethyl cellulose in water. Urine, faeces, tissues, expired CO2 and organic volatiles were collected. Animals were sacrificed after 7 days.
The study is accepted.

- Dermal absorption of ^{14}C Ziram in male rats: pilot dermal absorption study of ^{14}C Ziram in male rats (Cheng, 1991, Task force study)
Guidelines :
Experimental protocol of the study partially in compliance with test method B. directive 87/302/EEC or OECD guideline 417,1984.
Deviation from the official protocol: Distribution was not investigated.
GLP status: attest of study director and QAU not signed.
Material and methods :
A single dose of 1.1, 12 or 91 mg/animal of Ziram (specific activity: 13.9 mCi/mmol; 96.9%) was applied to the skin of 24 rats/dose (Crl:CD® (SD)BR) and was covered with a nonocclusive cover. Urine, faeces, blood , carcass and skin were collected. Animals were sacrificed at 0.5, 1, 2, 4, 10 and 24 hours after treatment.
The doses correspond to 0.086, 0.95, 7.25 mg/cm^2(or $\pm$ 5, 54.5, 413.6 mg/kg bw).
Skin wash : after removing of cell cover and enclosure of the dosing site, the skin was clamped to the interior of a funnel and washed with approximatively 100 ml of 1% soap solution into a tared-weight container that had approximately 150 ml 0.5% CMC solution. The skin wash solution was stirred for at least 1.5 hours.
Skin enclosure : the skin enclosure was soaked in 0.5% CMC solution for 2 days.
Skin : skin sample was weighed and digestetd with 1N NaOH for 3 days
The study is accepted.

Findings:

- Absorption:

Absorption was deduced from urinary and expired air excretion (table B.5.1-1, Cheng, 1989, task force study).
Absorption of Ziram from the gastro-intestinal tract is important in rats , reaching approximatively 71-75% after repeated low dose, 60-69% after high dose , and 58-61% after low dose administration within 48 h after oral administration. A mean absorption of 66% is proposed after oral administration.
Absorption is rapid after low single dose or repeated dose, reaching 50-70% of the dose within 24 h post-dosing. After high single dose administration a period of 48 h was necessary to obtain an absorption of 60-69% of the administered dose.

A dermal absorption study in rats showed that after low, medium or high dose application, absorption of radioactivity was 24.7, 26.3 or 3.37% respectively ; 76.2%, 68.7% and 99.9% of radioactivity was recovered in

samples of skin wash, skin cell cover and skin enclosure. Mean adsorbed radioactivity in the skin was 5.02, 5.91 or 1.17% for a low , medium or high dose and suggested that skin adsorption/absorption was not related to the dose applied.
In this study, nonaccountable radioactivity was high at low and medium dose representing respectively 19 and 26% of the administered dose and might have been eliminated in the expired air. (table B.5.1-2).
(Cheng, 1991, task force study)

- Distribution:

Distribution has not been studied in details : quite low levels of radioactivity (1.1-0.01%) were retained 7 days after oral administration and localised in the excretion organs (liver, kidneys, lungs), the carcass and blood.
Recovery of radioactivity was < 0.01% in bone, brain, fat, muscle, gonads, spleen, pituitary gland and thyroid gland.
After repeated exposure, recovery of radioactivity in tissues and carcass was low 7 days after exposure, suggesting that the compound does not accumulate (table B.5.1-3) (Cheng, 1989, task force study).

- Metabolism:

No data from the notifier. An experimental protocol was proposed in april 1997.

According to the JMPR (1996), Ziram is metabolized to dimethyldithiocarbamate and excreted as S-glucuronide conjugate. Other metabolic products include carbon disulfide, sulfate and dimethylamine (fig.5.1-1)

- Excretion:

After oral administration of ^{14}C-ziram to rats at dose levels of 15 or 352 mg/kg bw, the majority of the radioactivity was eliminated in urine (17% to 35%), feces (9% to 18%) and expired air (36% to 53%). The rate of elimination was relatively fast, the majority of the radioactivity was eliminated within 24 hours after dosing for the low dose groups, and within 48 h for the high dose group. No apparent sex-related differences were noted in ^{14}C elimination.
The mean recovered radioactivity ranged from 79 to 92% within 7 days from which low levels were retained in tissues and carcass (1.11 to 1.92%) table B.5.1-1 (Cheng, 1989, task force study).

Table B.5.1-1 : Excretion of ^{14}C-Ziram after oral exposure of rats as a function of time (Cheng, 1989, task force study)

Collection time	**% ^{14}C-recovery**					
dose mg/kg (frequency)	**15 (1x)**		**15(14x + 1x ^{14}C)**		**352**	
	–	–	–	–	–	–
faecal excretion						
0-24 h	12.65	5.01	8.41	8.59	1.45	6.47
24-48 h	1.81	5.67	2.32	2.23	4.36	6.80
total (0-168 h)	18.43	15.29	12.50	12.24	9.03	18.25
urinary excretion						
0-24 h	21.4	16.1	29.37	28.39	3.64	2.84
24-48 h	2.22	6.25	2.37	4.69	12.42	10.55
total (0-168 h)	26.34	25.46	32.6	35.41	22.61	17.46
expired air excretion						
recovery in activated charcoals traps						
0-24 h	15.69	15.48	13.76	20.52	25.71	21.71
24-48 h	0.06	0.11	0.07	0.1	4.74	6.47
total (0-96 h)	15.75	15.61	13.83	20.63	30.53	28.3
Recovery in CO_2 traps						
0-24 h	20.71	19.11	25.26	21.22	10.39	9.16
24-48 h	0.63	1.31	0.69	0.74	11.82	9.67
total (0-96 h)	21.70	20.70	26.20	22.18	22.68	19.28
Total radioactivity in expired air (0-96 h)	37.5	36.3	40	42.8	53.2	47.6
Total absorbed dose (0-24 h)*	57.8	50.7	68.4	70.1	39.7	33.7
Total absorbed dose (0-48 h)*	60.7	58.4	71.5	75.6	68.7	60.4
Tissues+carcass	1.64	1.88	1.68	1.92	1.12	1.25
Total mean recovery	**83.9**	**78.9**	**86.8**	**92.4**	**86**	**84.6**

* Absorbed dose = urinary excretion + expired air excretion.
Radioactivity in expired air was measured during 96 h.

Table B.5.1-2 : Recovery of ^{14}C-Ziram after dermal exposure of rats as a function of time (Cheng, 1991, task force study)

Collection time (h)	**% ^{14}C-recovery**		
mg/animal	**1.07**	**11.9**	**90.6**
Not absorbed dose:			
1. skin rinse			
0.5 h	75.22	72.39	101.96
1	85.35	69.03	99.79
2	78.9	67.03	99.73
4	74.64	60.71	97.20
10	70.07	72.33	100.26
24 h	70.53	68.79	93.49
2. skin enclosure			
0.5 h	0.10	0.42	1.02
1 h	0.14	0.17	1.44
2 h	0.33	0.31	1.02
4 h	0.20	0.17	0.22
10 h	0.07	0.09	1.03
24 h	0.35	0.43	1.26
3. cell cover			
0.5 h	0.06	0.02	0.04
1 h	0.08	0.04	0.06
2 h	0.12	0.05	0.18
4 h	0.12	0.06	0.26
10 h	0.26	0.14	0.15
24 h	0.60	0.16	0.35
Mean value (**E**skin rinse+ skin enclosure + cell cover)	**76.2%**	**68.7%**	**99.9%**
Adsorbed dose onto skin (test site)			
0.5 h	5.63	3.04	0.56
1 h	3.18	3.48	0.93
2 h	4.57	5.67	1.11
4 h	5.05	9.93	1.48
10 h	7.88	8.86	1.44
24 h	3.79	4.46	1.52
Mean value	**5.02%**	**5.91%**	**1.17%**
material balance*	**81.3±4.6%**	**74.6±3.7%**	**101±2.6%**
Absorbed dose :			
urine			
0.5 - 1 h	nd	nd	nd
2 h	<0.01	<0.01	nd
4 h	0.02	<0.01	<0.01
10 h	0.06	0.02	<0.01
24 h	0.16	0.05	<0.01
carcass			
0.5 - 4 h	nd	nd	nd
24 h	0.14	0.03	nd
blood			
0.5 h	0.002	nd	nd
24 h	0.006	nd	nd
feces	nd	nd	nd
nonaccountable	24.4	26.2	3.37

Collection time (h)	% ^{14}C-recovery		
mg/animal	**1.07**	**11.9**	**90.6**
Total absorbed dose: (Σ urine+carcass+nonaccountable+blood + skin adsorbed dose)			
	29.7 %	**32.19**	**4.55**

* mean recovery includes cage wash and cage wipe wch are minimal and therefore, not explained in the table.

Table B.5.1-3 : ^{14}C recovery in rat tissues and carcass 7 days after exposure (Cheng, 1989, task force study)

	recovered tissue radioactivity (% of administered dose)					
dose - mg/kg bw (frequency)	**15(1x)**		**15 (14x + 1 x ^{14}C)**		**352(1x)**	
sex	_	_	_	_	_	_
blood	0.22	0.39	0.25	0.34	0.24	0.32
carcass	0.89	0.99	1.00	1.11	0.70	0.73
heart	0.02	0.02	<0.01	0.02	<0.01	<0.01
kidney	0.05	0.06	0.05	0.06	0.02	0.03
liver	0.45	0.38	0.36	0.35	0.14	0.14
lung	0.02	0.03	0.03	0.03	<0.01	0.02
total	1.64	1.87	1.68	1.92	1.12	1.25

Conclusion:
In rats, Ziram is well absorbed from the gastro-intestinal tract, for 58-61% after a single low dose, for 60-69% after a single high dose and for 71-75% after a repeated low dose within 48 h after oral administration. Which means an overall oral absorption of 66%.

Partial inactivation, by a first-pass hepatic elimination, is suggested by the fact that, after inhalation, signs of systemic neurotoxicity were observed after 30 times lower doses than after oral ingestion.

After repeated exposure, recovery of radioactivity in tissues and carcass was low 7 days after exposure, suggesting that the compound does not accumulate.
In the dossier, metabolites were not identified and no metabolic scheme was proposed by the notifier. This point has to be addressed by the notifier. According to the JMPR (1996), Ziram is metabolized to dimethyldithiocarbamate and excreted as S-glucuronide conjugate. Other metabolic products include carbon disulfide, sulfate and dimethylamine .

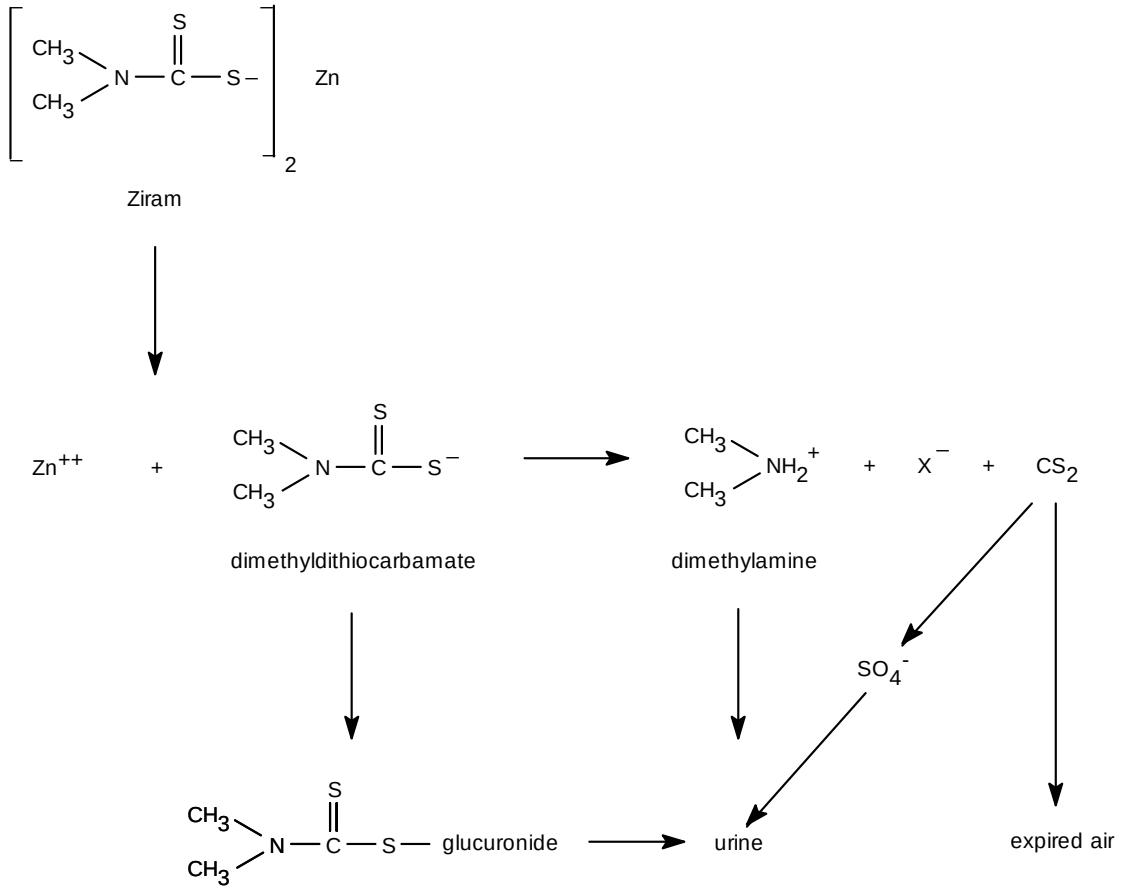
The most important elimination route of the radioactive marker was via expired air (36 to 53% of the dose administered), the majority of which was expired during the first 48 h post-dosing. Urinary excretion represented 17% (high dose within 24 h) to 35% (repeated low dose within 24 - 48 h). Faecal excretion represented 9 to 18.5% and decreased with increasing the dose.

Metabolism of Ziram in livestock:
Two lactating **goats** received an oral dose of 11.9 or 13.2 mg/kg bw each morning for 6 days. The occurrence of radiolabeled lactose and caseine identified in milk and radiolabeled urea in urine, indicates thet the metabolism of Ziram in goats occurs at least in part via a single-carbon biochemical pathway, which results in extensive radiolabeling of natural products (Bodden, 1993, task force study).

Metabolism of Ziram in plants:
Ziram was sprayed once at five times the USA recommended field rate (34 kg/ha). The major amount of Ziram remained on the surface of the **apples** and leaves and did not penetrate into the apples or leaves. Under wet conditions, Ziram dissociates to dimethyldithiocarbamate and is then broken down to volatile compounds such as CS_2. CS_2 related residues were 1.3% of the total residue at harvest (80 days post-treatment) ; although no metabolites

were identified, a similar pattern occurred in leaf as compared to apple . Substantial amounts of radioactivity were released during enzymatic treatment indicating again a participation in the single-carbon biosynthetic pathway. (Wyss-Benz, 1994, task force study).

Figure B.5.1-1 : Expected metabolic pathways of ziram, on the basis of data on other dimethyldithiocarbamates (JMPR, 1996)



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

B.5.2.1 Acute oral toxicity (Annex IIA 5.2.1)

- Rat, oral administration by gavage, 250, 400 or 640 mg/kg bw (Liggett and Allan, 1989a, task force study)

Findings :
Mortality: deaths occurred from day 2 to day 3 at all dose levels: at 250 mg/kg (1_, 2_); at 400 mg/kg (3_, 4_); at 640 mg/kg bw(4_, 5_);
Body weight: losses for all rats that died. Low bodyweight gain were recorded for 1 male and 1 female at 250 mg/kg bw and 1 male at 640 mg/kg on day 8.
Clinical signs: piloerection was observed in all rats within five minutes of dosing accompanied by hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities and diarrhoea in all rats at later intervals on day 1 .
Recovery was complete by day 3 or 5.
Necropsy: no deviation from normal morphology was found.
Conclusion :
Clinical signs suggest that ziram , at high doses, is toxic for the central and autonomic nervous system.
LD_{50} male: 381 (227-594) mg/kg bw
LD_{50} female: 267 (113-394) mg/kg bw
LD_{50} combined: 320 (176-422) mg/kg bw

Guidelines:
Experimental protocol not fully in compliance with the test B.1- 1bis of directive 92/69/ EEC.
GLP status : yes (no attest of competent authority)
Deviation from the official protocol: absence of control group.
Material and methods :
5 CD rats (Crl : CD(SD) BR VAF plus) /sex/dose received by gavage a single dose of an aqueous suspension of Ziram (98.5%; B.n°8331)in 1%w/v aqueous methyl cellulose at 250, 400 or 640 mg/kg bw.
The study is accepted.

B.5.2.2 Acute percutaneous toxicity (Annex IIA 5.2.2)

- Rabbit, 2000 mg/kg, 24 hours, occlusive patch (Liggett and Allan, 1989b, task force study)

Findings :
Mortality: no deaths were noted.
Clinical signs: no signs of systemic toxicity
Dermal response: slight erythema in 2 male and 2 females. The reaction had resolved completely by day 3 or 4 of the study.
Bodyweight: a loss of body weight (5%) was observed in 2 females on day 8.
Necropsy: 1 male presented pale renal cortices at post-mortem.

Conclusion :
LD_{50} > 2000 mg/kg

Guidelines:
Experimental protocol fully in compliance with the test B.3 of Directive 92/69/EEC .
GLP status : yes (no attest of competent authority)
Material and methods :
Limit test:
5 New Zealand white rabbits /sex were exposed to 2000mg/kg Ziram (98.5%; b.n°.8331) by dermal occlusive application during 24 h.
The study is accepted.

B.5.2.3 Acute inhalation toxicity (Annex IIA5.2.3.)

- inhalation, rats, 4-hour whole body exposure , dust, 0.02, 0.029, 0.047, 0.098, and 0.145 mg/l (Jackson and Hardy, 1989, task force study)

Findings :

Mortality: 0.047 mg/l : 0/5_,4/5_ ; 0.098 mg/l : 2/5_ ,4/5_ ; 0.145 mg/l : 5/5_,4/5_. Death occurred within 2 days post exposure.
Clinical signs:
During exposure: partial closing of eyes, irregular respiration rate. Some animals had reduced motor activity, salivation, a reduced respiration rate, exaggerated respiratory movements and piloerection.
During observation period: abnormal respiration persisted for up to 8 days following exposure, dark appearance of eyes and lethargy.
Recovery was complete within 9 days following exposure.
Body weight: reduced body weight or rate of bw gain for up 2 days following exposure.
Food and water consumption were reduced for up to 2 days following exposure. Some female rats had a high water consumption during the observation period.
Necropsy: in animals that died, the relative lung weight were increased and congested, occasional swollen appearance of the lungs and gas-filled stomachs. Some animals presented a frothy fluid in the trachea.

Conclusion :
LC_{50} male : 0.08 mg/l $\left(\frac{\text{LC50 mg/l x 7 l/h x 1000 mg x 4 hours}}{333} = 6.72 \text{ mg/kg bw}\right)$
LC_{50} female : 0.06 mg/l (5.04 mg/kg bw)
LC_{50} combined: 0.07 mg/l (5.88 mg/kg bw)

Guidelines :
Experimental protocol in compliance with the method B.2 ,annex V of Directive 92/69/EEC.
GLP status : yes (no attest of competent authority)
Material and methods:
10 Sprague-Dawley rats (CHbb:THOM(SPF))/sex/dose were exposed for 4 h by a whole body inhalation exposure of a test atmosphere containing dust generated from Ziram at 0.02, 0.029, 0.047, 0.098, and 0.145mg/l (98.5%;B.n°8331 AA) (MMAD: 1.4-2.3 µm ; mean respirable % :90.6-76.4%)
The study is accepted.

- inhalation, rats, 4-hour nose only exposure , dust, 0.96, 0.52, 0.1, and 0.06 mg/l (Blagden 1991, task force study)

Findings :

Mortality: 0.06 mg/l : 0/5_; males not exposed; 0.10 mg/l : 0/5_,4/5_ ; 0.52 mg/l : 5/5_, no_ exposed ; 0.96 mg/l : 5/5_,5/5_;

Clinical signs:
During exposure: decreased respiratory rate, wet fur.
Males exposed to 0.52mg/l in addition to incidents of laboured and noisy respiration, hypothermia, pallor of extremities, increased salivation and red/brown stains around the snout presented hunched posture, lethargy, pilo-erection, gasping respiration, ataxia and ptosis.
3 Animals which were removed from exposure at 0.96 mg/l showed signs of hunched posture, lethargy, pilo-erection, gasping respiration, ataxia and ptosis.
During observation period: Animals exposed to 0.06 mg/l showed wet fur, hunched posture, lethargy, pilo-erection, decreased respiration rate and ptosis. Isolated incidents of ataxia and red/brown stains around the snout. Recovery within 2 days after exposure.
Animals exposed to 0.1 mg/l showed hunched posture, pilo-erection, decreased respiratory rate, ptosis (1 male). Incidents of ataxia, lethargy and red/brown stains around the snout and isolated incidents of laboured respiration, noisy respiration, pallor of extremities. Surviving animals appeared normal 2 to 7 days after exposure.
Body weight: normal in surviving animals.
Necropsy: in animals that died or were killed in extremis, haemorrhagic and swollen lungs. Abnormal redness and

presence of fluid in lungs and some dark livers. Congestion of small intestine (at 0.1 mg/l) and reddening of the small intestine (at 0.96 mg/l). Gaseous distension of small intestine. Surviving animals showed no abnormalities at necropsy.

Conclusion :
LC_{50} male : 0.18 mg/l (15.13 mg/kg bw)
LC_{50} female : 0.08 mg/l (6.72 mg/kg bw)
LC_{50} combined: 0.13 mg/l (10.93 mg/kg bw)

Guidelines :
Experimental protocol in compliance with the method B.2, annex V of Directive 92/69/EEC.
GLP status : yes (attest of competent authority is from 1989; the study was performed in 1991).
Material and methods:
10 Sprague-Dawley rats (Charles River))/sex/dose were exposed for 4 h by a nose only inhalation exposure of a test atmosphere containing dust generated from Ziram at 0.96, 0.52 (males only), 0.1, and 0.06mg/l (females only) (98.4%;l ot n°.1208 AB/V609) (MMAD: 1.3-1.7 µm ; mean respirable% :97.1-88.2%)
The study is accepted.

- inhalation, rats, 4-hour nose only exposure , 0.20, 1.15 or 4.96 mg/l , aerosol (Kieran, 1995, Bakelite)

Findings:
Mortality : at 0.2 mg/l 1/5_ and 1/5_ were found dead within 4 h of exposure . A further 3/5 _and 3/5 _ were found dead within 24 h after exposure. The remaining 1/5 _ and 1/5 _ survived.
At 1.15 mg/l (5/5 _ and 1/5 _) died within 3 h or less exposure ; the remaining 4/5 _ were found dead within 24 h after exposure.
Dead occurred within 3h or less of exposure at 4.96 mg/l (5/5 _and 2/5 _), the remaining 3/5 females (4.96 mg/l) were found dead within 2 h after exposure.
Clinical signs:
Common to all groups, intensity generally concentration related: slow deep/laboured breathing, subdued behaviour, hunched posture and unsteady gait.
Body weight: marked losses in decedent animals and in surviving animals from the low dose but this group recovered after 7 days of observation.
Necropsy: for premature decedents, swollen and inflamed lungs and a clear trachea discharge were reported. Swollen stomachs were also observed and resulted probably from swallowing air during exposure. In surviving animals, no significant findings were reported.
Relative lung weight was markedly increased in the 3 exposed groups, and this was considered to be due to a marked pumonary oedema resulting from exposure.
Conclusion: surviving animals recovered within 7 days of observation.
LC_{50} < 0.2 mg/l (= 16.81 mg/kg bw)

Guidelines :
Experimental protocol partly in compliance with the method B.2, annex V of Directive 92/69/EEC.
GLP status : :no
Material and methods:
5 Sprague-Dawley rats (Charles River))/sex/dose were exposed for 4 h by a nose only inhalation exposure of a test atmosphere containing Ziram (97.4%; lot n°.4/52019) as a powder aerosol at 0.20,1.15 or 4.96 mg/l. (MMAD: 2.54, 3.69, 5.49 µm).
Deviation from the official protocol: exposure time is reduced to 2 h40 consecutively to the high mortality. Different batches of animals were used consecutively to mortality and the incorporation of additional exposure
The study is accepted.

B.5.2.4 Skin irritation (Annex IIA 5.2.4)

- 6 Rabbit, semi occluded dermal exposure, 0.5 g (Liggett and McRae, 1990a, task force study)

Findings :

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

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

Conclusion :
Ziram is not irritating.

Guidelines:
Experimental protocol in compliance to the test B.4. of Directive 92/69/EEC.
GLP status : The study is GLP (no attest of competent authority).
Material and methods :
6 male New Zealand white rabbits were exposed to 0.5 g of Ziram (98.5%; b.n°.8331A) applied to the intact skin and covered with a semi-occlusive dressing during 4 h.
The study is accepted.

- 3 Rabbit, 0.5g, occlusive patch (Haynes and Brightwell, 1995a, Bakelite)

Findings:

$<Se>_{24+48+72\ h} = 0\ /0/0$

$<So>_{24+48+72\ h} = 0/0/0$

Conclusion :
Ziram is not irritating.

Guidelines:
Experimental protocol in compliance with the test B.3 of Directive 92/69/EEC .
GLP status : yes (no attest of competent authority)
Material and methods :
3 female New Zealand white rabbits were exposed to 0.5 g Ziram (97.4 %; b.n°.4/52019) by dermal occlusive application during 4 h.
The study is accepted.

B.5.2.5 Eye irritation (Annex IIA 5.2.5)

- Eye irritation , 1 rabbit (Liggett and McRae, 1990b, task force study)

Findings:
Due to the severity of the response, only 1 animal was exposed and the study was terminated 24 hours after instillation.

Table B.5.2.5-1 : Ocular reactions elicited by Ziram

region of eye	one hour	1 day after instillation	result
cornea	A	3	+
iris	A	2	+
conjunctivae: redness	2	3B	+
chemosis	4	4	+
A: unable to assess due to degree of chemosis B: necrosis and partial destruction of nictitating membrane			

An opacity covering the entire corneal surface was present 24 hour after instillation. Iridial inflammation had developed by the 24 h reading. A diffuse beefy-red colouration of the conjunctivae accompanied by considerable swelling with the eyelids more than half-closed and a copious discharge 24 h following instillation. Necrosis and partial destruction of the nictitating membrane were also present.
Conclusion :
Ziram is a severe ocular irritant.

Guidelines:
Experimental protocol in compliance with the test B.5, annex V, of Directives 92/69/EEC or 84/449/EEC or OECD guideline 405 (1981-87).
GLP status : yes (no attest of competent authority)
Material and methods :
80 mg (0.1 ml) of Ziram (98.5%; b.n°.8331A) was instillated into the lower everted lid of one eye of 1 New Zealand white rabbit (_).
The study is accepted**.**

- Eye irritation , 3 rabbits (Haynes and Brightwell, 1995b, Bakelite)

Findings:
The study was terminated at 24 h , the response being regarded as causing unacceptable pain and distress to the animals. There was no indication of a systemic effect of treatment.

Table B.5.2.5-2 : Ocular reactions elicited by Ziram - Individual scores

region of eye	one hour	1 day after instillation
cornea opacity	1/1/1	2/2/2
area involved	2/2/3	4/4/4
iris inflammation	1/1/1	1/1/1
conjunctivae: redness	2/2/2	3/3/3 + necrosis of lower palpebral conjunctiva
discharge	3/1/2	3/3/3
chemosis	3/3/3	2/3/3

Conclusion: Ziram has severe irritant effect in the eye.

Guidelines:
Experimental protocol in compliance with the test B.5, annex V, of Directives 92/69/EEC or 84/449/EEC or OECD guideline 405 (1981-87).
GLP status : yes (no attest of competent authority)
Material and methods :
100 Mg (0.1 ml) of Ziram (97.4 %; b.n°.4/52019) was instillated into the lower lid of one eye of 3 New Zealand white rabbits

(&). Animals were examined 1 and 24 h after instillation. The study is accepted.

B.5.2.6 Skin sensitization (Annex IIA 5.2.6)

- Skin sensitizing potential in guinea pigs (Split Adjuvant Test) (Daamen, 1988, task force study)

Findings :
A primary irritation test was carried out using 5 guinea pigs to determine the concentrations to be applied in the challenge phase. Solutions at 50, 25, 10 and 5% of Ziram was applied for 24 h under occluded patch.

Six animals showed a positive response (grade 2 or more) in reaction to the 10% concentration. Five of these animals reacted also positively in response to the 5% concentration and 3 of these animals also positively in response to the 1% solution.
A total of 14/20 animals had a score ≥ 1

Conclusion :
Ziram is a sensitizer.

Guidelines:
The test to be carried out in accordance with dir. 92/69/EEC method B.6(84/449/EEC) is the method Maximization test which is the preferred technique. The Split Adjuvant Test was used .
Deviation from the official protocol: The duration of induction period was 9 days and not 8 as proposed in the appendix of method B.6, dir. 84/449/EEC. The challenge was realized with one exposure and not 2.
GLP status : yes (no attest of competent authority)
Material and methods :
20 female Guinea-pigs (Dunkin Hartley) were induced by epicutaneous applications of Ziram, 25% in corn oil (99.04%, b.n°.8331 AA). Challenge with a series of test substance concentrations (10, 5 and 1% w/w) in corn oil . 10 animals served as control group.
The study is accepted.

- Guinea pig, Maximization test (Haynes and Brightwell, 1995c, Bakelite)

Findings :
The preliminary tolerance test indicated that ziram at 0.02% was reasonably tolerated by the test system. The topical application test demonstrated that the test substance at the highest concentration achievable (75%) was tolerated , exhibiting no irritant response.
In the main study, induction phase, significant reaction was apparent at the sites of injection following administration of Freund's complete adjuvant alone (test and control group) , mixed with the compound or the compound alone. Sutes treated with the substance (test group) exhibited a mild response and those sites treated with the vehicle alone (control group) exhibited little response to the treatment. All signs of irritation around the site of injection were observed after 48 h.
Challenge phase: 2 animals died during the study and reaction of the test substance was observed in 2/18 surviving animals of the test group , 24 h after completion of the challenge dosing procedure. In meantime, two animals of the control group exhibited reaction to the substance at 24 h. After 48 h no animals of either treated group and control group showed signs of irritation.
Examination of the animals that died during the study revealed changes to the lungs. These effects reflect more probably the health status of the animals and are probably not consecutive to treatment with ziram.
Conclusion :
Ziram is not a sensitiser.

Guidelines:
Protocol in compliance with method B.6 of Directive 84/449/EEC.
GLP status : yes
Material and methods :
In the dose-ranging study, 2 animals were injected intradermally with the substance at 20%, 10%, 5%, 2%, 1% and 0.5% in sterile water. A second group of 2 animals were treated with the test substance diluted with an emulsion of Freund's complete adjuvant. Necrosis was observed at all treated skin sites within 24 h of dosing in the dose-ranging screen and this phase of the study was repeated with lower doses of 0.5, 0.2, 0.1, 0.05, 0.02, 0.01% in bothe sterile water and an emulsion of Freund's complete adjuvant.

Topical application tolerance test : 5 animals were injected intradermally with Freund's complete adjuvant. Each animal was dosed with 75, 50, 20, 10 or 5% in sterile water.
Main study: 20 female Guinea-pigs (Dunkin Hartley strain) received an intradermal induction by injection of Ziram (97.4 %; b.n°.4/52019) as a 0.02% solution in sterile water. Percutaneous induction of 75 % test substance as a paste in sterile water. For the challenge reaction, the animals were exposed dermally to 75 % test substance as a paste in sterile water, 21 days after the intradermal induction.
A second group with 10 animals served as a control group.

B.5.2.7 Summary of acute toxicity of Ziram (Annex IIA 5.2)

Ziram is harmful when swallowed, and very toxic by inhalation. It is not a skin irritant but produces severe ocular lesions. Skin sensitization may occur.
Partial inactivation by a first-pass hepatic elimination, is suggested by the fact that, after inhalation (5.88 mg/kg bw), signs of systemic neurotoxicity were observed after 50 times lower doses than after oral ingestion (320 mg/kg bw). At doses of 300 and 600 mg/kg bw, single oral administration (see point B.5.7.1), clear neurotoxicity was observed, affecting the autonomic, sensorimotor and central nervous systems. Mild signs of neuromuscular and CNS dysfunction were, however, already observed at 15 mg/kg.
It has been suggested that these neurotoxic effects might be due to the metabolite CS2 (Hardy et al., 1998).

Target organs, as indicated by macroscopic changes , are liver and lungs.

Table B.5.2.7-1 : Summary of acute toxicity of Ziram

Type of test	Result (mg/kg bw)	Purity (%)	Classification	Reference
LD_{50} oral rat1% _ _ + _	381 (227-594) mg/kg bw 267 (113-394) mg/kg bw 320(176-422) mg/kg bw	98.5% b.n°.8331AA	**Xn, R22**	Liggett and Allan, 1989a, task force study
LD_{50} skin rabbit	> 2000 mg/kg bw	98.5% b.n°.8331AA	-	Liggett and Allan, 1989b, task force study
LC_{50} rat, 4 h dust, whole body _ _ _ + _	0.08 mg/l (6.72 mg/kg bw) 0.06 mg/l (5.04 mg/kg bw) 0.07 mg/l (5.88 mg/kg bw)	98.5% b.n°.8331AA	**T^+ R26**	Jackson and Hardy, 1989, task force study
LC_{50} rat, 4 h dust, nose only _ _ _ + _	0.18 mg/l (15.13 mg/kg bw) 0.08 mg/l (6.72 mg/kg bw) 0.13 mg/l (10.93 mg/kg bw)	98.4%;lot n°.1208 AB/V609	**T^+ R26**	Blagden, 1991, task force study
LC_{50} rat, 4 h aerosol, nose only _+ _	< 0.2 mg/l (16.81 mg/kg bw)	97.4%; lot n°.4/52019	-	Kieran, 1995, Bakelite
Skin irritation rabbit 4 h	not irritant	98.5%; b.n°.8331A	-	Liggett and McRae, 1990, task force study
Skin irritation rabbit 4 h	not irritant	97.4 %; b.n°.4/52019	-	Haynes and Brightwell, 1995a, Bakelite
Eye irritation rabbit	severe irritant	98.5%; b.n°.8331A	**Xi, R41**	Liggett and McRae, 1990b, task force study
Eye irritation rabbit	severe irritant	97.4 %; b.n°.4/52019	-	Haynes and Brightwell, 1995b, Bakelite
Split adjuvant test	sensitizer	99.04%, b.n°.8331 AA	**R43**	Daamen, 1988, task force study
Skin sensitization guinea pig MK	not a sensitizer	97.4 %; b.n°.4/52019	-	Haynes and Brightwell, 1995c, Bakelite

B.5.3 Short term toxicity (Annex IIA 5.3)

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

- Rat, oral administration, 3, 15, 100 mg/kg bw/d by gavage, 4 weeks (Dickhaus and Heisler, 1980, task force study)

Findings :
No particular clinical signs nor deaths were reported.
Food efficiency was reduced and the effect was dose-related in male and females; males and females of the high dose-group had significantly reduced food consumption and body weight was significantly reduced in males (100 mg/kg) and in females(15 and 100 mg/kg).
Haematological results were normal.
At 100 mg/kg, blood total albumin was increased in females and Alkaline Phosphatase (AP) was increased in male and female.
Urinalysis was normal.
At necropsy, at 15 mg/kg bw, partly patch patterns of liver with slightly swollen marginal zone. These effects were also noted at 100 mg/kg bw : kidneys presented a gravely surface , spleens were slightly enlarged and liver weight were increased.
Histopathological examination showed degenerative alterations of the liver and the kidney tubuli in group 15 and 100 mg/kg and extramedullary hematopoiesis in the range of red pulpar cells of the spleen at 100 mg/kg.

Conclusion :
NOAEL = 3 mg/kg bw not taking into account the marginal reduction in food efficiency reported in this group.

Guidelines:
Experimental protocol not fully in compliance with the test B.7 of Directives 92/69/EEC.
Deviation from the official protocol: haematological examinations were realized on 5/10 rats /dose and urinalysis on 3/10 rats.
Clotting potential not controled.
GLP status : no
Material and methods :
15 Wistar rats/sex/group were administerd Ziram (98%) at concentrations of 0, 3, 15 and 100 mg/kg bw over a period of 4 week.
The study is accepted.

- Rats, 4 weeks, 60, 180, 540 ppm (2.5 è 34 mg/kg bw/d)- (satellite group from the 104 week study) (Powell et al., 1994a, Task force study)

Findings:
Measurement of T3, T4 and TSH revealed a dosage related reduction in T3 and T4 values at week 4. In paralel, TSH was increased.
Table B.5.3.1-1 : Oral 4 week toxicity of ziram- satellite animals from the 104 week study

Endpoints/dose	0 ppm		60 ppm		180 ppm		540 ppm	
Clinical chemistry	–	–	–	–	–	–	–	–
T3 (ng/dl)	49	47	47	42	44	44	45	40
T4 (µg/dl)	3.7	2.7	3.4	2.4	3*	2.4	2.8*	2.0*
TSH (ng/dl)	5.7	4.7	8	4.7	7.6	4.9	6.9	4.1

*statistically significantly different from control.

Material and methods:
The results reported here were obtained from the 10 male and 10 female rats used in the 104 week study in rats (point B.5.5.3) in which blood was taken from the orbital sinus in week 4.
50 Crl:CD(SD)BR rats/sex/dose received in the diet , ziram (technical; 98.7%; b.n°.8331A) at dosage levels of 60, 180 or 540 ppm for 104 weeks.
Converted doses in males week 1-104: 2.5; 7.7 or 23.7 mg/kg bw/day.
females: 3.4; 10.2 or 34.6 mg/kg bw/day.

- Rat, oral administration, 500, 1000, 2000 ppm (45 è 170 mg/kg bw/d) in the diet, 4 weeks (Chambers et al., 1992a, task force study)

Findings :
No deaths were reported.
Hairloss was noted among the majority of females receiving 500 and 1000 ppm.
At 2000 ppm, this effect was generalized in all animals and majority of females were emaciated. Alopecia was noted in 4/5 males and 5/5 females receiving 2000 ppm and in 2/5 males receiving 1000 ppm. This was occasionally associated with staining.
Treated males showed an increasing dosage-related trend towards lower than control body weight gain. For all treated males and females, there was a trend of reduced food consumption.
All treated males had increased relative liver weight and decreased absolute liver weight .
An apparent reduction in brain and pituitary weights among treated rats, reduced testes weights and uterus weights were considered to be associated with their lower body weights.

Conclusion: the lowest dose of 45.5 mg/kg bw elicits toxic effects and a NOAEL cannot be defined.

Guidelines:
Experimental protocol not fully in compliance with the test B.7 of Directives 92/69/EEC.
Deviation from the official protocol: haematological examinations was not performed. Histopathological examination was not performed on preserved organs and tissues. The lowest dose was too high. The study is a range-fonding study.
GLP status : no
Material and methods :
5 Charles River rats (Crl:CD(ICR)BR) sex/group received Ziram (98.5%; b.n°8331 AA) in the diet at 500, 1000 or 2000 ppm over a period of 4 weeks.
Chemical analysis of trial diets indicated a 26.3% reduction of the a.s. concentration after 8 days at room temperature. The high level concentration of 5000 ppm did not show any significant change in concentration over a similar period.
Achieved intake: 45.9, 89.6, 170 mg/kg bw/day for males
45.5, 84.7, 170 mg/kg bw for females
The study is accepted.

- Mice, oral administration, 3000, 4000, 5000 ppm (374 è 776 mg/kg bw /d) in diet, 4 weeks (Chambers et al., 1992b, task force study)

Findings:
No deaths were reported. No abnormal clinical signs.
The overall body weight gain of male and females was markedly lower in all treated groups. There was a generalized lowering of food consumption for animals receiving Ziram and efficiency of food utilisation was impaired as compared to the controls.
Absolute brain and heart weight was reduced in all dosed females and absolute heart weight in male dosed 5000 ppm .
Macroscopic examination revealed minimal adipose tissue in 2/5 male and 2/5 female treated with 5000 ppm and 1/5 female treated with 4000 ppm and 1/5 male treated with 3000 ppm

Conclusion: signs of systemic toxicity are observed in all treated groups. No NO(A)EL in this study.

Guidelines:
Experimental protocol not fully in compliance with the test B.7 of Directives 92/69/EEC.
Deviation from official protocol:
Haematology was not made. Histopathological examination was not performed on preserved organs and tissues. The lowest dose was too high.
GLP status : no
Material and methods :
5 Charles River mice (Crl:CD(ICR)BR)sex/group received in the diet Ziram (98.5% ; b.n°8331 AA) at concentrations of 3000, 4000 and 5000 ppm over a period of 4 week.
Achieved intake: 374, 514, 736 mg/kg bw/day for males
424, 656, 776 mg/kg bw for females
The study is accepted.

- Dog, oral administration, 1000, 2000, 5000 ppm in diet, 4 weeks (McLean et al, 1992a, task force study)

Findings:
At 1000 ppm (± 25 mg/kg bw/day), one female vomited on 1 occasion. Body weight and food consumption were nearly normal. Liver weight exceeded the normal upper limit of 4.0% of bodyweight.
At 2000 ppm (± 50 mg/kg bw/day), one female was killed following convulsion episodes.
Male vomited and had diarrhea occasionally. Body weight and food consumption were nearly normal. Liver weight exceeded the normal upper limit of 4.0% of bodyweight.
Macroscopic post-mortem examination showed in female a red streak on aortic valve, congestion of cervical lymph node and in male, foci of red mucosal congestion in jejunum.
At 5000 ppm (± 125 mg/kg bw/day), male and female were killed following inappetence, loss of body weight and/or deterioration in clinical conditions.
Animals vomited and had diarrhea occasionally. Body weight and food consumption were slightly reduced. Liver weight exceeded the normal upper limit of 4.0% of bodyweight. Subdued behaviour and/or unsteady gait.
Macroscopic post-mortem examination showed pale colour of thyroid, pallor of muscles, fluid cyst on ovary and red discolouration on left lobe of lungs.

Conclusion: in this study the lowest dose is not really a NOAEL because some clinical signs suggest a toxic effect.

Guidelines:
Experimental protocol not fully in compliance with the test B.7 of Directives 92/69/EEC.
Deviation from official protocol:
Haematology was not made. Histopathological examination was not performed on preserved organs and tissues.
GLP status : no
Material and methods :
2 beagle dogs (pure bred) /group received in the diet Ziram (98.5% ; b.n°8331 AA) at concentrations of 1000, 2000 and 5000 ppm over a period of 4 week.
The study is accepted.

B.5.3.2.1 Oral 90 day toxicity (rat) (Annex IIA 5.3.2)

- Rat, dietary administration of 100, 300, or 1000 ppm (7.4 è 79 mg/kg bw/d), 13 weeks (Powell et al., 1992, task force study)

Findings: The lowest dose elicited toxic signs and was therefore, too high.

Table B.5.3.2-1 : Oral 90 day toxicity in rats

Endpoint/dose	**0**		**100 ppm**		**300 ppm**		**1000 ppm**	
	_	_	_	_	_	_	_	_
Mortality		1						
Clinical signs					hairloss			
Food consumption					î13%	î17%	î25%	î25%
Food efficiency								impaired wk 1
Body weight gain					î18%	î18%	î33%	î32%
Ophthalmoscopy			no lesions indicative of a reaction to treatment					
Haematology								
RBC							î	î
MCHC					ì	ì	ì	ì
Clinical chemistry								
urea nitrogen				ì		ì	ì	ì
total protein				î		î		î
serum Ca^{2+}			î	î	î	î	î	î
serum AP								ì
organ weight (r)								
brain					ì	ì	ì	ì
spleen						ì	ì	ì
testes or ovary					ì		ì	ì

Statistically significant increase (ì) or decrease (î) Fisher's exact analysis, Barlett's test, one way analysis of variance and Kruskal-Wallis analysis of ranks, Student's 't' test and Williams test.

Other findings: localised areas of minimal epithelial hyperplasia of the non-glandular stomach, proximal to the limiting ridge, were observed in 3/10 females and 1/10 males at 1000 ppm and also in 1/10 female at 300 ppm. Similar changes were absent from controls. The change observed was minimal and probably as a result of minor irritation produced on the mucosa. This effect was not statistically significant .

Conclusion :
NOAEL < 7.4 mg/kg bw/day.

Guidelines :
Experimental protocol not fully in compliance with the test method B. of Directive 87/302/EEC or OECD guideline 408(1981).
Deviation from the official protocol: the experimental animals were not observed daily after week 4; histopathology was carried out on a limited number of organs.
GLP status : yes (except for competent authority attest)
Material and methods :
10 Charles River rats (Crl:CD(SD)BR)sex/dose received in the diet Ziram (98.7% ; b.n°8331 AA) at concentrations of 100, 300 or 1000 ppm over a period of 13 week.
Achieved intake: 7.4, 21.4, 67.8 mg/kg bw/day fro males
8.8, 24.2, 76.9 mg/kg bw for females
The study is accepted.

- Rats, oral administration , 13 weeks, 60, 180, 540 ppm- (satellite group from the 104 week study) (Powell et al., 1994a, Task force study)

Findings:
No animals died. Food consumption and body weight were reduced at the top dose, but no statistical analysis was performed on these data.
Full biochemical investigation in week 13 are showed in table B.5.3.2-2.

Table B.5.3.2-2 : Oral 13 week toxicity of ziram- satellite animals from the 104 week study

Endpoints/dose	**0**		**60 ppm**		**180 ppm**		**540 ppm**	
	–	–	–	–	–	–	–	–
Body weight (g)	485	282	490	283	477	275	428	252
Food consumption (g/week)	177	133	182	132	175	123	157	118
Haematology								
Hb (g/dl)	15.3	13.8	14.9	14.3	14.9	14.2	14.6*	14.7
RBC ($x10^6/mm^3$)	7.6	6.9	7.3	6.8	7.2*	6.7	7.1*	6.7
MCHC (%)	30.3	28.7	29.8	29.9	29.9	30.2*	29.9	30.3*
MCV(fl)	67	70	68	71	69*	71	69*	72*
Thrombotest (s)	27	20	25	19	23*	19	23*	19*
Clinical chemistry								
Glucose (mg/dl)	97	116	108*	113	109*	115	117*	115
Total protein (g/dl)	6.9	7	6.7	7.1	6.5*	6.9	6.5*	6.7*
Albumin (g/dl)	3	3.5	3	3.4	2.9*	3.3	2.8*	3.1*
Urea nitrogen (mg/dl)	14	19	16	20	16	22	17*	23
Creatinine(mg/dl)	0.5	0.6	0.6	0.7	0.5	0.7	0.6*	0.6
Ca^{++} (mEq/l)	5.4	5.4	5.5	5.4	5.4	5.3*	5.2*	5.1*
ALT (mU/ml)	30	29	29	29	23*	29	22*	22
T3 (ng/dl)	44	62	43	56	39	60	45	55
T4 (µg/dl)	3.7	2.9	3.4	2.7	3.2	2.7	3.2	2.6
TSH (ng/dl)	10.4	6.3	8.6	6.1	9.4	6.2	11.6	6.8

*statistically significantly different from control.

Conclusion:
Minor but consistent changes in biochemical parameters occurred in both male and female rats receiving 180 or 540 ppm. At 60 ppm, values were within the expected ranges and changes were considered not to represent toxicological significance.
Not taking into account the slight glucose increase at 60 ppm, the NOAEL in this study = 60 ppm = 3mg/kg bw/day.

Material and methods :
20 Crl:CD(SD)BR rats/sex/dose received in the diet , ziram (technical; 98.7%; b.n°.8331A) at dosage levels of 60, 180 or 540 ppm for104 weeks. During week 13, samples of blood were withdrawn from the orbital sinus of 10 male and 10 female rats from each group.
Converted doses in males week 1-52: 3; 9.1 or 27.3 mg/kg bw/day.
females: 3.9; 11.7 or 37.5 mg/kg bw/day.

- Mice, oral administration in diet , 100, 300, 900, 2700 ppm, 13 weeks (Powell et al., 1993, Task force study).

Findings :
The lowest dose elicited toxic signs and was therefore, too high.
Major findings are reported in table B.5.3.2-3 and are considered to be treatment-related signs . Minimal hyperkeratosis of the non-glandular epithelium of the stomach in exposed mice as weel as the minimal epithelial hyperplasia of the limiting ridge were considered to be of minor toxicological significance.

Table B.5.3.2-3 : Oral 90 day toxicity in mice

Endpoint/dose	**0**		**100 ppm**		**300 ppm**		**900 ppm**		**2700 ppm**	
	_	_	_	_	_	_	_	_	_	_
Mortality					1		2			
Clinical signs							half closed sunken eyes		swollen dark abdomen	
Body weight gain				î (33%)		î (32%)	î (30%)	î (31%)	î (58%)	î (63%)
Food consumption			î (13%)		î (17%)	î (21%)	î (26%)	î (28%)	î (35%)	î (30%)
Food efficiency									Impaired (wk 1)	
Organ weight:										
thyroid (r)										ì
liver(r)										ì
spleen						î	î	î	î	î
brain								î		î
heart							î		î	
uterus										î
Histopathology:										
small spleen		0/10						2/10	2/10	
contracted spleen		1/10	1/10				4/10		5/10	3/10
ovaries : corpora lutea:								absent in 2/10		absent in 2/10
thin uterus		0/10						1/10		2/10
hyperkeratosis non glandular epithelium stomach:	1/10	1/10					ì 4/10	ì 3/10	ì 10/10	ì (8/10)
minimal epithelial hyperplasia of limiting ridge in stomach:									ì2/10	ì2/10

Statistically significant increase (ì) or decrease (î) Bartlett's test, one way analysis, Kruskal-Wallis analysis of ranks; Analysis of variances by Student's 't' test and Williams'test; (r) = relative organ weight

Conclusion : minor changes in the stomach, spleen and female reproductive tract in mice are reported after treatment with 2700 or 900 ppm. Important decreased body weight gain and food consumption was noted at 100 ppm making this dose a toxic dose. A NOAEL cannot therefore, be established in this study.

Guidelines :
Experimental protocol partly in compliance with the test method B. of Directive 87/302/EEC or OECD guideline 408 (1981).
Deviation from the official protocol: the experimental animals were not observed daily after week 4; haematology , clinical biochemistry, and ophtalmological examination were not investigated ; histopathology was carried out on a limited number of organs.
GLP status: yes (except for competent authority attest)

Material and methods :
10 Charles River mice (Crl:CD(ICR)BR) sex/dose received in the diet Ziram (98.7% ; b.n°8331 AA) at concentrations of 100, 300, 900 or 2700 ppm over a period of 13 week.
Achieved intake: 15, 44, 125, 364 mg/kg bw/day for males
19, 49, 134, 410 mg/kg bw for females
The study is accepted.

B.5.3.2.2 Oral 90 day toxicity (dog) (Annex IIA 5.3.2)

- Dog, oral administration in diet, 100, 300, 1000 ppm in diet (McLean et al., 1992b, Task force study)

Findings :

Table B.5.3.2.2-1 : Oral 13 week toxicity in dogs

Endpoint/dose	**0**		**100 ppm**		**300 ppm**		**1000 ppm**	
	_	_	_	_	_	_	_	_
Mortality							1	
Clinical signs							convulsion	trembling
Food consumption			satisfactory					
Body weight gain							î 42%	
Haematology								
APTT*					ì		ì	
Clinical chemistry								
AP							ì	ì
Cholesterol					ì	ì	ì	ì
Globulin			ì	ì	ì	ì	ì	ì
Albumin			î	î	î	î	î	î
Urinalysis			no effects related to Ziram					
Organ weights								
absolute liver (no stats.)				ì (8%)	ì (9%)	ì (10%)	ì (15%)	ì (17%)
heart absolute							î	
lung absolute								î

Statistically significant increase (ì) or decrease (î) Williams's-test ; Bartlett's test ; one-way analysis of variance and Kruskall-Wallis of ranks. 1,2,3, : number animals involved.
*: APTT :activated partial thromboplastin time

At 1000 ppm : treatment was considered to be associated with a slight lowering of group mean red cell indices (PVC, Hb, RBC) in males and females at week 6 and 13, although these differences were not always statistically significant. Slightly elevated circulating reticulocyte counts, with associated polychromasia, hypochromasia and /or moderate anisocytosis, were noted in some animals in week 6. Slightly elevated reticulocyte count was also apparent for 1 dog at week 13. 1 Dog was killed for humane reasons: the animal had a seizure from which recovery was not apparent.Prior to seizure, the dog had convulsive episodes, inappetence and body weight loss. Heart changes were observed likely to be related to the convulsions. The centrilobular degeneration of hepatocyte was possibly secondary to heart changes seen.
At week 5, individual serum AP, AST, CPK and cholesterol values were markedly higher, while serum ALT, Na^+ and Cl^- levels slightly higher than the pre-dose values. A high urinary specific gravity and protein was noted. Liver weight was increased, spleen and prostate weight were lower.
The liver of female presented multiple depressed pale areas. Focal necrosis, together with loss of cells and dilated sinusoids correlated with the high individual AP, ALT, AST and OCT noted for this dog.
At 300 or 1000 ppm, minimal amounts of pigment were seen in the Kupffer cells. These changes were not seen in control dogs. Focal necrosis was also seen in the male dog at 300 ppm.

Acute inflammatory cell infiltration in lungs was noted in 1 dog at 1000 ppm associated with minimal foci of eosinophilic homogenous material. The latter effect was also noted in 2 dogs at 100 ppm and 1 dog at 300 ppm. The toxicological significance of this finding is uncertain.

Conclusion: liver is the target organ as suggested by the focal necrosis and the appearance of pigmented Kuppfer cells.
NOAEL = 4.07 mg/kg bw not taking into account the reduced blood protein level and the liver weight increase observed in female dogs.

Guidelines :
Experimental protocol in compliance with the test method B. of Directive 87/302/EEC or OECD guideline 408(1981).
Deviation from the official protocol: The highest dose level produced 1 fatalitiy and was therefore, too high.
GLP status : yes (no attest of competent authority)
Material and methods :
4 pure-bred beagle dogs/sex/dose received in the diet Ziram (98.7% ; b.n°8331 AA) at concentrations of 100, 300, or 1000 ppm over a period of 13 weeks.
Achieved intake: 4.07, 12.15, 41.81 mg/kg bw/day for males
4.31, 13.04, 41.07 mg/kg bw for females
The study is accepted.

B.5.3.2.3 Oral 1 year toxicity (dog) (Annex IIA 5.3.2)

-Dog, oral administration in diet, 50, 185 or 500 ppm (Smith et al., 1993, Task force study)

Findings *:* The MTD was reached.

B.5.3.2.3-1 : 52-Week dietary chronic toxicity study with Ziram in dogs

Endpoints/dose	**0**		**50 ppm**		**185 ppm**		**500 ppm**	
	_	_	_	_	_	_	_	_
Mortality							1	
Clinical signs:					no effect of treatment			
Body weight gain						î68%		î81%
Food consumption					no effect of treatment			
Ophtalmoscopy					no effect of treatment			
Haematology:					no effect of treatment			
Clinical chemistry								
albumin				î12%		î16%	î13%	î16%
cholesterol							ì51%	
T3							ì33%	
Organ weight							ì 2/4	
liver							16%	
Histopathology of liver:								
foci degenerated hepatocytes	1/4	3/4	2/4	0/4	2/4	3/4	3/4	2/3
aggregates of pigmented Kupffer cells	0/4	0/4	1/4	1/4	2/4	4/4	4/4	2/3
Spleen:								
ìpigmented macrophages	1/4	2/4	1/4	2/4	3/4	2/4	3/4	3/3

Statistically significant increase (ì) or decrease (î)($p \leq 0.05$) Bartlett's test; Kruskal-Wallis of ranks ; Student's 't'test; Fisher's exact and Mantel'test; Williams'test.

In the high-dose group, the dose was reduced from 700 ppm to 500 ppm in week 12 following a treatment-related mortality. The dog being found in a collapsed state with excessive salivation, trembling, champing of the jaws and

jerking of the limbs. Another dog was killed following incidence of suspected polyarteritis, which was unrelated to treatment. This animal has been replaced by another in the study.
Biochemistry analysis revealed intergroup differences in ALT values amongst males (not statistically significant) : 1 dog (185 ppm) and 2 dogs (500 ppm) showed elevated ALT in week 26 and 52. Since these animals showed the most marked changes in the liver microscopic examination, it was considered that these changes were treatment-related.
AP values showed an apparent dosage-related increase amongst males receiving 185 or 500 ppm in week 26 and 52, although differences were not statistically significant but considered as a result of treatment and are probably associated with the pathological findings seen in the liver of these dogs at termination.
The decrease in albumin was not supported by any significant corroborative pathological finding at termination and was considered of equivocal toxicological importance.
A slight increased T3 value recorded for males at 500 ppm did not form part of a dosage related trend, there was no clear dosage-related trends in T4 values, individual data were generally within the historical control range and in the absence of any effect on thyroid weight or morphology, these differences were considered of no importance.

There were no histopathological changes in the livers of dogs with higher liver weights.

At 500 ppm, group mean heart weights were decreased compared with controls, the differences attaining statistical significance for combined male and female data. Group mean prostate weights showed a dosage-related decrease compared with the control data, although the differences did not attain statistical significance. There were no macro- or microscopic findings for these tissues.

Macroscopic pathology : multiple red discoloured linear depressions of gastric mucosa for 1 female at 185 ppm and a single oval, flat friable choleliths in the gall bladders of 2 males at 185 ppm. No similar findings were seen in any dog at the 500 ppm treatment level.

Histopathology: liver and spleen are the target organs. Foci of degenerate hepatocytes were seen to a moderate degree at 500 ppm and at 185 ppm with a single cell necrosis in one male at 500 ppm. Inflammatory cell infiltration around central veins and sometimes around the branches of the hepatic vein were seen in some dogs. An apparent increase in centrilobular fibrocytes was seen in 3/4 male dogs treated with 500 ppm and in 1/4 male dog treated with 185 ppm. There was an increased incidence and degree of aggregates of pigmented Kupffer cells and macrophages in the livers of male and female dogs and an increased incidence of pigmented macrophages in the spleen of male dogs treated with 500 ppm or 185 ppm when compared to control male dogs.
The initial process involved in the changes of these tissues appeared to be increased pigmentation of Kupffer cells and /or macrophages with Perls' positive material, possibly reflecting increased iron metabolism and turnover. These changes were evident at all dosages in the liver, and for males at 185 or 500 ppm in the spleen. At 185 and 500 ppm, more marked associated changes were seen in the liver, chiefly hepatocyte degeneration and inflammatory cell infiltration. The only change identified at the low dose that could be possibly related to treatment, was aggregates of pigmented Kupffer cells and macrophages in one male and one female.

Conclusions:
NOAEL: 50 ppm = 1.6 mg/kg bw/day

Guidelines:
Protocol in compliance with method B of Directive 87/302/EEC or OECD 452 (1981).
GLP status : yes (no attest of competent authority)
Material and methods :
4 Beagle dogs/sex/dose received 0, 50, 185 or 500 ppm ziram (b.n°. 8331AA, 98.5%) in their diet during 52 weeks.
Converted dose: 1.6, 6.6, 17.4 mg/kg bw/d for males.
1.9, 6.7, 20.6 mg/kg bw/d for females.
The study is accepted.

B.5.3.3.1 28-day inhalation toxicity (rat) (Annex IIA 5.3.3)

No data. Not necessary.

B.5.3.3.2 90-day inhalation toxicity (rat) (Annex IIA 5.3.3)

No data. Not necessary.

B.5.3.3.3 Percutaneous 21-day toxicity (rat) (Annex IIA 5.3.3)

-Rabbit, intact skin, 100, 300, 1000 mg/kg bw/d, 21 days (Edwards et al.,1989, Task force study)

Findings :
There were no clinical signs related to treatment. No dermal reactions to treatment were observed amongst the rabbits. Transient cases of staining of the dose site were recorded amongst animals of group 1000 mg/kg bw/d.

At 1000 mg/kg bw/d:
Decreased body weight gain and food consumption were observed in females which were statistically significant.
Lymphocyte counts were statistically significantly lower for female rabbits and considered to be treatment-related.
Liver enzymes AST and ALT were significantly increased, and bilirubine were increased in females and cholesterol was increased in male and females.
At 300 mg/kg, the occurrence of a real effect on liver enzymes was supported by significantly increased AST for females.
Organ weights were normal. No abnormal macroscopic or microscopic findings were observed.

Conclusion*:*
NOAEL= 100 mg/kg bw/d

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(no attest of competent authority)
Material and methods :
Ziram (98.5%; b.n°: 8331AA) was applied daily for 6 h to the clipped intact dorsal skin under occluded patch of 8 New Zealand white rabbits/sex/dose over a period of 3 weeks at a dose of 100, 300 or 1000mg/kg bw.
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.
The study is accepted.

B.5.3.3.4 90-day dermal toxicity (rat) (Annex IIA 5.3.3)

No data. Not necessary

B.5.3.3.5 Summary of the short-term toxicity studies of Ziram (Annex IIA 5.3.3)

In rats and mice ziram decreased, body weight gain, food intake and food efficiency. Dogs presented a subdued behaviour and/or unsteady gait, with slight alterations in body weight and food efficiency, increase in liver weight, they vomited and had diarrhoea.

In the rat, T4 serum level showed a dose related reduction, T3 and TSH were not significantly affected. Moreover, minor, but consistent, changes in biochemical parameters were observed (îtotal protein , albumin, Ca^{++}, RBC and ìurea nitrogen , creatinine and MCHC).

In the rat, the target organs were liver and kidneys, as shown by the presence of degenerative alterations. In mice, toxic effects were more diffuse and no specific target organ could be determined. Dogs showed an increased liver weight.

In the subchronic experiment (90 day study), the non-glandular stomach was the target organ, with minimal epithelial hyperplasia in rats, and epithelial hyperkeratosis and minimal epithelial hyperplasia of the limiting ridge in the stomach of mice.No effects indicative of neurotoxicity were apparent when FOB tests were performed in the rat. In dogs, the target organs were liver and spleen, with at the start increased pigmentation of Kupffer cells and/or macrophages with Perls'positive material, possibly reflecting increased iron metabolism and turnover. In dogs, also the following observations were made, a slight lowering of red cell indices, acute inflammatory cell infiltration in the lungs, decreases in heart weight, likely related to convulsions.

In these studies (rat, mice, dog), the lowest doses administered already produced adverse effects, which were sometimes quite minimal but consistent with the observations made at higher doses.

In a 21-day percutaneous test, ziram affected the liver in rabbits. This effect occurred at 300 mg/kg bw/d. No dermal reactions were observed.

Table B.5.3.3.5-1 : Summary of the short-term toxicity studies of Ziram

Study	**NOAEL** mg/kg bw/day	**LOAEL** mg/kg/day	**Critical effects**	**Purity, b.n°.**	**Reference**
28 day , oral rat	3	15	î body weight, î food efficiency degenerative alterations in liver and kidney tubuli	98%; unknown batch	Dickhaus and Heisler, 1980 task force study
28 day , oral rat	<45.5	45.5	î body weight gain; ì liver weight (r) ;î food consumption	98.5% 8331 AA	Chambers et al., 1992a task force study
28 day , oral mice	<374	374	î body weight gain, îfood consumption, î heart weight, î brain weight	98.5% 8331 AA	Chambers et al., 1992b task force study
28 day , oral dog	<25	25	vomiting, ìliver weight	98.5% 8331 AA	McLean et al, 1992a task force study
90 day , oral, rat	< 7.4	7.4	ì BUN, îCa^{2+} ,î blood proteins	98.7%; 8331 AA	Powell et al., 1992 task force study
90 day , oral, rat (satellite group 104 wk)	3	9.1	î RBC, î blood protein, îalbumin ìMCV	98.7%; b.n°.8331A	Powell et al., 1994a, Task force study
90 days, oral, rat neurotox.	systemic tox. :14 neurotox.: 34	34 -	î mean brain neurotoxic esterase -	V528/8331AA; 97.87%	Lamb, 1993 task force study
90 day , oral, mice	<15	15	î body weight gain, îfood consumption, î spleen weight	98.7%; 8331 AA	Powell et al., 1993 task force study
90 day , oral, dog	4.07	12.15	liver necrosis, pigmented Kupffer cells	98.7%; 8331 AA	McLean et al, 1992b task force study
1 year , oral, dog	1.6	6.6	hepatocyte degeneration, inflammatory cell infiltration	8331AA, 98.5%	Smith et al., 1993 task force study
21 day, dermal rabbit	100	300	ì AST in females	98.5%: 8331 AA	Edwards et al;1989 task force study

B.5.4 Genotoxicity (Annex IIA 5.4)

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

B.5.4.1.1 Gene mutation test in bacterial cells (Annex IIA 5.4.1)

- Ames Salmonella/mammalian -microsome mutagenicity (Jones et al., 1990, Task force study).

Findings :
In the plate incorporation assay, doses up to 50µg/plate gave negative results with and without S9 mix with the exception of strain TA100 when, at 50 µg/plate, the reversion rate was twofold increased in the presence of S9 mix. Therefore, in order to confirm the data, experiments were performed using various concentrations of S9 mix with strain TA100. A borderline positive response was noted. Preincubation in liquid medium did not increase the response.
A positive dose-response relationship was noted when higher concentrations of Ziram were used (50, 75, 100µg/plate) with higher concentrations of S9 mix (20 or 30%).

Conclusion : Ziram is mutagenic towards *S.typhimurium* in the presence of metabolic activation .

Guidelines:
Experimental protocol fully in compliance with method B 14 of directive 92/69/EEC. This study was performed in good experimental conditions; the criterias for the determination of a positive response are well defined and appropriate. Positive controls are appropriates .
GLP status : yes (no attest of competent authority)
Material and methods :
Salmonella strains TA 98, TA 100, TA 1535, TA 1537, TA1538 were used.
The standard plate incorporation assay was realized using 5000, 500, 50, 5 µg/plate of (98.5%; B.n°8331 AA) for the dose-range finding study; 50, 15, 5, 1.5, 0.5 µg/plate for the first mutation experimentation with and without S9mix. This experiment was repeated.
An other assay was performed in order to increase the sensitivity: preincubation in liquid medium was associated to the use of different concentrations of S9mix (5, 10 or 20% S9 fraction in the mix) with strain TA100. In this assay, the following concentrations of Ziram were used: 150, 100, 50, 25, 15 µg/plate (Aroclor 1254 induced rat liver).
Using TA 100, an assay was performed where the S9conc. was raised to 20 and 30% without preincubation. The doses were 100, 75, 50, 25_µg/plate Ziram.
Each experimental point was performed in triplicate .
The solvent used was DMSO. A negative control was realized with the solvent.
positive control + S9 : 2-aminoanthracene
positive control without S9: N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and nitrofluorene.
The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate. Positive controls gave the expected response.
The study is accepted.

B.5.4.1.2 Gene mutation test in mammalian cells (Annex IIA 5.4.1)

No data. This study is necessary to confirm the positive results obtained in bacteria.

B.5.4.1.3 *In vitro* chromosome aberration assay on human lymphocytes (Annex IIA 5.4.1)

- *In vitro* mammalian cytogenetic test using CHO-K1 cells (Brooker and Akhurst, 1989, Task force study)

Findings :
In the absence of metabolic activation, Ziram was highly toxic at all dose levels, with very few metaphase figures present in cultures treated with the lower dose levels and no live cells present in cultures treated with the 4 highest dose levels (5 to 40 µg/ml). The test was therefore repeated using a lower range of dose levels from 0.8 to 400 ng/ml. In this repeat test a dose dependent decrease in mitotic index was seen at the 5 highest dose levels and with 25 ng/ml giving a mitotic index which was 22.4% of the solvent control value. No statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations at any dose level when compared with the solvent control.

In the presence of S_9, the 6 highest dose levels (1.3 to 40 µg/ml), were highly toxic. In the repeat test. In this assay, the second highest dose of 1000 ng/ml proved to be sufficiently toxic by producing a mitotic index which was 52.9% of the solvent control value.The highest dose was excessively toxic. No statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations at any dose level when compared with the solvent control.

Conclusion :
Ziram is not clastogenic in these experimental conditions.

Guidelines :
Experimental protocol not fully in compliance with method B10 of dir. 92/69/EEC or 84/449/EEC.
Deviation from the official protocol: at least duplicate cultures must be used for each experimental point. The repeat study did not use the same concentrations. Only one harvest time was used.
GLP status : yes (no attest of competent authority).
Material and methods :
Chinese hamster ovary cells, strain K1-BH4 were obtained from Bibra.
Ziram (98.5%; B.n°.8331 AA) dissolved in DMSO was used at final concentrations of 0.08, 0.2, 0.3, 0.6, 1.3, 2.5, 5, 10, 20 and 40_µg/ml and in the presense of metabolic activation at 0.01, 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.3, 2.5, 5, 10 20 and 40 µg/ml.. Cultures without S9 were incubated 21 h. In the presence of S9 , the medium was removed after 4 h and incubation was continued for 17 h.
The assay in the absence of S9 was repeated with 0.8, 1.6, 3.1, 6.3, 12.5, 25, 50, 100, 200 and 400 ng/ml because the higher concentrations were toxic. In the repeat study with S9, the following doses were used: 5, 50, 125, 250, 375, 500, 750, 1000 and 1500 ng/ml..
The highest doses tested inhibited mitotic activity
Positive controls -S9 : mitomycin C
Positive controls +S9 : cyclophosphamide
Negative controls : DMSO. A negative control without solvent was not performed. The sensitivity of the test was demonstrated as well as the efficacy of the S9 mix. The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate.
The study is accepted.

B.5.4.1.4 *In vitro* unscheduled DNA synthesis in mammalian cells (Annex IIA 5.4.1)

- *In vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes (Proudlock, 1989, Task force study)

Findings :
Doses of 3160 ng/ml and above proved toxic resulting in cell death and sloughing of cells from the coverslip.
The positive control (AAF) caused large, dose-related highly significant increases in the net nuclear grain together with substantial increases in the gross nuclear grain count.
In neither test did Ziram cause a statistically significant increase in the net nuclear grain count.

Conclusion :
Ziram did not induce DNA repair in this test.

Guidelines:
Experimental protocol not fully in compliance with method B of annex V (dir. 87/302/EEC)or test 482(OECD guideline,1986) .
Deviation from the official protocol: the area count mode was used and the count obtained converted to an equivalent grain count using a conversion factor. Where a strong positive response was obvious only 25 nuclei/cytoplasmic areas were compared .
GLP status : yes (no attest of competent authority).
Material and methods : Ziram (98.5%, b.n°.8331AA) disssolved in DMSO was tested from 0.316 ng/ml up to 100 µg/ml . This upper concentration gave a noticeable precipitate in the culture medium.
2-Acetylaminofluorene was used as positive control.
Hepatocytes were isolated from male Albino Sprague-Dawley rats. A suspension of hepatocytes was prepared. Triplicate cultures were used at each experimental point.The culture was treated with the compound or solvent and incubated for 20 h (first exp) and 19 h (second exp.). UDS was quantificated by autoradiography.
The study is accepted.

B.5.4.2. *In vivo* genotoxicity testing (somatic cells) (Annex IIA5.4.2)

B.5.4.2.1. *In vivo* mammalian bone-marrow micronucleus test (Annex IIA 5.4.2).

- Mouse, 25, 75, 225, 675 ppm in diet during 89 day, peripheral blood micronucleus test (Proudlock and Taylor, 1992, Task force study)

Findings:
Ziram did not cause any statistical significant increases in the number of micronucleated polychromatic erythrocytes nor in the incidence of micronucleated normochromatic erythrocytes. There was no decrease in the ratio of polychromatic to normochromatic erythrocytes. In this study, the MTD was reached.
Conclusion:
Ziram did not show any evidence of chromosome-damaging activity or bone-marrow cell toxicity in these *in vivo* conditions.

Guidelines: Protocol not fully in compliance with the test method B.12 of draft EEC(1997) and OECD TG 474, mammalian erythrocyte micronucleus test (1996).
GLPstatus: yes (no attest of competent authority)
Material and methods:
Deviation from the official protocol: a positive control was not included .
5 CD-1 outbred albino mice received in the diet 25, 75, 225, 675 ppm Ziram (98.8%; 8331AA) corresponding to 5, 15, 45, 135 mg/kg bw/d. After 89 days exposure, peripheral blood smears were prepared.
The study is accepted.

- Mouse, 125 or 250 mg/kg bw for male and 150 or 300 mg/kg bw for females by gavage, micronucleus test (Ciliutti and Brightwell, 1995, Bakelite)

Findings:
Following treatment with ziram, slight increases in the ratio of mature polychromatic erythrocytes were observed in male group at both dose-levels at the 24 h sampling time, indicating that the test substance exerted a slight toxic effect on the bone-marrow cells. A slight increase was also observed in female group at the high-dose level at the 48 h sampling time.
Slight increases , not statistically significant, in the incidence of micronucleated PCE's were observed at the 24 h sampling time at both dose-levels. No other increases were noted.

Conclusion: Under the reported experimental conditions, ziram administered orally at dose-levels of 125 and 250 mg/kg bw to males and 150 and 300 mg/kg bw to females does not induce micronuclei in the polychromatic erythrocytes of treated mice.

Guidelines: Protocol not fully in compliance with the method B.12 of Directive 92/69/EEC.
GLPstatus: yes (no attest of competent authority)
Material and methods:
5 CD-1 albino mice received orally 125 or 250 mg/kg for males and 150 or 300 mg/kg for females Ziram (97.4%; batch 4/52019). The vehicle used was 0.5% carboxymethylcellulose in sterile water. Mitomycin-C served as positive control.
Deviation from the official protocol: only one sampling time was taken for the positive control.
The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate.
Positive controls gave the expected response.
The study is accepted.

B.5.4.2.2. *In vivo* mammalian bone-marrow chromosome aberration test (Annex IIA 5.4.2).

- Mouse, oral single dose administration, 40, 120 or 400 mg/kg (Völkner, 1992a, Task force study)

Findings:
In a pre-experiment, 4 mice received orally a single dose of 200, 300, 400, 600, or 700 mg/kg bw of Ziram and on the basis of results of toxicity, 400 mg/kg was chosen as maximum dose for the mutagenicity assay. At this dose,1 male and 1 female died at preparation interval 24 h and 1 male died at preparation interval 48 h and this dose was the MTD.
Mitotic index: 3.52% (400 mg/kg bw) ; 4.03% (40 mg/kg bw) and 5.5% (120mg/kg bw).
At no preparation interval the chromosome aberration frequency was substantially enhanced as compared to the negative control.
Carboxymethylcellulose was used as negative control. Cyclophosphamide, used as positive control showed a distinct increase over the spontaneous aberration frequency.

Conclusion: Under the experimental conditions reported, ziram did not induce chromosome aberrations in bone marrow cells of mouse.

Guidelines: Protocol in compliance with the test method B.11 of directive 92/69/EEC.
GLP status: yes
Material and methods: Ziram (98.4%, b.n°.8331 AA/V528) was suspended in CMC(0.5%) and a single oral dose of 40, 120 or 400 mg/kg was administered to 6 mice (NMRI)/sex/dose. Animals were killed 24 h after treatment. Additional sacrifices were performed in the high dose group at 6 and 48 h. Negative and positive controls were killed at 24 h post-treatment. 5 animals /sex/dose were evaluated. The study is performed under good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate. Positive controls gave the expected response.
The study is accepted.

B.5.4.2.3. *In vivo* unscheduled DNA synthesis test with mammalian liver cells (Annex IIA 5.4.2).

- Rat, single oral administration , gavage, 500, 1000 or 2000 mg/kg bw (Falezza and Brightweel, 1995, Bakelite)

Findings *:*
Ziram was not cytotoxic for the hepatocytes in the concentrations tested. No increase in the UDS activity was observed in either of the concentrations tested. The number of cells in repair was similar for both control and ziram. 2-AAF induced a strong positive response in both experiments and the % of cells in repair was up to 92%.
Conclusion *:*
Ziram is considered to be inactive in the UDS assay under the given test conditions .

Guidelines:
Protocol in compliance with the OECD TG 486 (1996) or draft testing protocol from EC (1997)
GLP status : yes (no attest of competent authority)
Material and methods *:*
4 male Sprague-Dawley CD rats/dose received by gavage Ziram (97.4%;b.n° 4/52019) at 500; 1000; 2000 mg/kg bw in aqueous suspension (CMC 0.5%). Animals were sacrificed 14h after dosing in the first experiment and 2 hours after treatment in the second experiment and hepatocytes were isolated.
Positive control for UDS: 2-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.
The study is accepted.

B.5.4.3 *In vivo* studies in germ cells (Annex IIA 5.4.3)

B.5.4.3.1 Cytogenetic assay in mouse germ-cells (Annex IIA 5.4.3).

- Mouse, chromosome aberration in spermatogonial mitoses (Völkner, 1992b, Task force study)

Findings: The highest dose administered was determined in pre-experiments for toxicity to be the MTD. All treated animals expressed toxic reactions.
At the tested concentrations, mitotic index was 1.4% (200mg/kg) ; 2.34% (67 mg/kg); 1.4% (20 mg/kg); 0.34% (positive control). Treatment with Ziram did not result in a significant enhancement of the aberration frequency at any preparation interval or dose level tested as compared to the negative control value.

Conclusion: Ziram did not induce chromosome aberrations in spermatogonia of the mouse under these experimental conditions.

Guideline: The experimental protocol is fully in compliance with the test method B, annex V, dir 87/302/EEC or OCDE guideline 483(1986).
GLP status: yes
Material and method:
Ziram (98.4%; b.n°.8331 AA/V528) in suspension in CMC (0.5%) was administered orally as a single dose of 20, 67 or 200 mg/kg bw to 6 mice (NMRI from Charles River Wiga)/sex/dose. The rate of chromosome aberrations was examined 24 h after administration of the 3 doses ; additional sampling times of 6 and 48 h were added for the high dose. Cyclophosphamide was used as positive control. The study is performed in good experimental conditions; the acceptance and evaluation criterias are well defined and appropriate. Positive controls gave the expected response.
The study is accepted.

B.5.4.4 Additional information from open literature :

***In vitro* genotoxicity tests:**

1 . Tests for gene mutations:

Table B.5.4.4-1 : Tests for gene mutations *in vitro*

Strains, experimental conditions	Doses	Product, purity	Results	Reference
S. typhimurium TA1535, TA1537, TA98, TA100, TA102 plate incorporation assay with w/o S9 (rat) solvent : DMSO (C-) C+ were used 2 exp.	0, 3.75, 7.5, 15, 30, 60 90 µg/plate	tech. product (98.5 %), UCB	w/o w S9 : + in TA1535, TA100	Crebelli et al, 1992
*E. coli*WP2 (pKM101) WP2 uvrA (pKM101) plate incorporation assay with w/o S9 (rat) solvent : DMSO (C-) C+ were used 2 exp.	0, 3.75, 7.5, 15, 30, 60 90 µg/plate	tech. product (98.5 %), UCB	w/o S9 : + in WP2 uvrA (pKM101)	Crebelli et al, 1992
S. typhimurium TA1535, TA1537, TA98, TA100 preincubation procedure with w/o S9 (rat hamster) solvent : DMSO (C-) C+ were used	0, 3, 10, 33, 100 333 µg/plate	Uniroyal, 86.2 %	w/o w S9 : + in TA100	Haworth et al., 1983
S. typhimurium TA1535, TA1537, TA1538, TA98, TA100 plate incorporation assay with w/o S9 (rat) solvent : DMSO (C-) C+ were used	0, 5, 10, 50, 100 µg/plate	pure (recrystalized)	w/o w S9 : + in TA100, TA1535	Hedenstedt et al., 1979
L5178Y TK+/TK- mouse lymphoma w/o S9 solvent : DMSO (C-) C+ : EMS 2-exp	0.0625-2 µg/ml	unknown	positive from 0.0625 µg/ml (in presence of 79% cytotox., lethality at 1.8 µg/ml)	McGregor et al., 1988
HPRT in V79 cells w w/o S9 (rat) (2, 20 %) solvent : DMSO (C-) C+ :EMS DMN expo. : 4 h	0-0.08 µg/ml	unknown, rubber factories	negative	Donner et al., 1983

Results from open literature for induction of gene mutations *in vitro*, confirm the ability of ziram to induce point mutations in *S. typhimurium*. In mammalian cells, a positive mouse lymphoma test and a negative HPRT test in V79 cells. Both studies were realised with a compound of unknown purity.

2. Tests for chromosome damage :

Table B.5.4.4-2 : Tests for chromosome damage

Tissue, experimental conditions	Doses	Product purity	Results	Reference
Chromosome aberrations , CHO cells w w/o S9 (rat) solvent : DMSO (C-) C+ : MMC CP w/o S9 : expo.10 h, cell harvesting 2-3 h later w S9 : expo. 2 h, cell harvesting 11 h later w/o S9 : 2 exp., w S9 : 1 exp	w/o S9 : 0.01- 0.1 µg/ml w S9 : 1.5-1.75 µg/ml	Uniroyal Chemical, 86.2 %	w w/o S9 : positive	Gulati et al., 1989
Comments: important informations are lacking in this report ;scoring of positive results is not explained and only mentionned as positive or negative result ; no statistics.				
Chromosomal aberration CHO cells w w/o S9 (rat) C- : solvent = DMSO untreated C+ : MMC CP expo : w/o S9 : 24 h w S9 : 3 h sampling : at 24 h 200 metaphases /exp. point	- S9 : 0.001-0.046 µg/ml + S9 : 0.46-2.15 µg/ml	technical (99.7 %) UCB	w/o S9 : negative with S9 : conc. %CA MI solv. 3 100 1 10* 88 2.15 24* 61	Mossesso et al., 1994
Chromosomal aberration in CHEL w/o S9 C- : solvent = DMSO untreated C+ : DMBA expo : w/o S9 : 24 h det. MI, 200 metaphases anal./exp. point	0.22-1 µg/ml	technical (99.7 %) UCB	positive with S9 conc %CA solv. 2.5 0.22 7 0.46 8 1 13.5*	Mossesso et al., 1994
Comment: both studies reported positive results with S9 but the experiments are not replicated. *statistically significant				
Sister chromatid exchange test in CHO cells w w/o S9 (rat) solvent : DMSO (C-) C+ : MMC CP w/o S9 : expo.26 h, cell harvesting 2-3 h later w S9 : expo. 2 h, cell harvesting 26 h later w/o S9 : 2 exp., w S9 : 4 exp	w/o S9 : 0.001-0.025 µg/ml w S9 : 0.16-1.75 µg/ml	Uniroyal Chemical, 86.2 %	w w/o S9 : negative	Gulati et al., 1989

Data from open literature suggest the ability of ziram to induce chromosome aberrations in mammalian cells.

3. Other indicators of genetic damage :

Table B.5.4.4-3 : Repairable DNA damage in bacteria and recombinagenicity in yeast

Tissue, experimental conditions	Doses	Product purity	Results	Reference
Mitotic gene conversion *S. cerevisiae* D4	500/1000 ppm	Fuclasin-Ultra (trade name)	negative	Siebert et al., 1970
Rec-assay *B.subtilis* H17 Rec^+ M45 Rec^-	unknown	unknown	positive	Kada et al., 1980
Rec-assay *B.subtilis* H17 Rec^+ M45 Rec^- solvent : DMSO (C-)	0.6 µg/disk	Kodaira-shi, Japan	negative	Shirasu et al., 1976

The positive Rec-assay was realised with unknown doses and purity of the compound. The Rec-assay in *Bacillus subtilis* (measure of increase in mortality in recombinationless bacteria) detects DNA-damage chemicals, since different types of DNA damage are subjected to cellular recombination.
Many mutagens are active in the SCE assays, but it is still incompletely understood how DNA damage or perturbations of DNA synthesis give rise to SCE.

***In vivo* genotoxicity tests:**

1. Tests for gene mutations:

Table B.5.4.4-4 : Sex-linked recessive lethal test (gene mutations and small deletions in germ cells)

Tissue, Strain, Experimental conditions	Doses	Product, purity	Results	Reference
D. melanogaster expo. feeding 24 h solvent : ethanol-sucrose	0, 0.05, 0.25 0.5 mg/ml	unknown, Swedish Finnish rubber factories	positive	Donner et al., 1983
D. melanogaster treat. time : 48 h, 72 h 96 h	0, 1.3×10^{-2} - 7.8×10^{-2}	Cuman L* (trade name)	signif.- freq. sex-linked recessive lethals	Tripathy et al., 1989
D. melanogaster expo. feeding (24 h) solvent : ethanol-sucrose	unknown	unknown	positive	Donner et al., 1981
D. melanogaster expo. feeding (72 h) solvent : ethanol-sucrose	1000 ppm	Uniroyal, 86.2 %	positive	Foureman et al., 1994

* Cuman L contains 27% active substance and 73 % inert ingredients.

The results reported in table B.5.4.4-4 suggest that Ziram induces sex-linked recessive lethals in *Drosophila melanogaster*. The studies are lacking important informations such as the purity of the compound, the doses tested, absence of positive controls, absence of experimental protocol and no replication of experiments or limited exposure levels.

2. Tests for chromosome damage :

Table B.5.4.4-5 : Chromosomal aberrations and micronuclei

Strains , experimental conditions	Doses	Product, purity	Results	Reference
Chromosomal aberrations in germ cells Swiss Albino male mice (5 an/group) orl (gavage), 5 days sacrifice 19th day after last treat. analysis of 200 metaphases/an C- : solvent :dist. water C+ : EMS	0, 350, 700 1050 mg/kg (sublethal doses) (LD_{50} orl mice : 1400 mg/kg)	Cuman L **(trade name) Hindustan Ciba-Geigy	**positive** conc. % - 5.8 350 22.18* 700 20.79* 1050 18.40*	Hemavathi Krishnamurthy, 1988
Comment: 1 sampling time, no dose-response relationship and high spontaneous background				
Micronuclei in mouse (B6C3F1) BM, single i.p. exposure C- : DMSO C+ : hydroquinone sampling times : 24 & 48 h 2000 PCE/animal analysed NCE/PCE det.	_ : 2.5, 5 10 mg/kg _: 5, 10 20 mg/kg (preliminary toxicity : 50 mg/kg = all treated mice died, 20 mg/kg : 3/5 _ died all _ survived)	tech. product (98.5 %), UCB	**negative** at 24 48 h, even at cytotoxic conc. (stat signif ¯ % PCE at 48 h in _ 10 mg/kg _ 10 and 20 mg/kg)	Crebelli et al, 1992
Micronuclei in BM cells Swiss Albino male mice (5 an/group) orl (gavage), 2 split doses (0 h 24 h) sacrifice 6 h after last treat. 3000 PCE/animal analysed NCE/PCE det. C- : solvent :dist. water C+ : EMS	0, 350, 700 1050 mg/kg	Cuman L **(trade name) Hindustan Ciba-Geigy	**positive** dose %MN - 0.01 350 0.2* 700 0.11* 1050 0.06*	Hemavathi Krishnamurthy, 1988
Comment: only one sampling time and moreover too short; unknown purity ; no clear dose-response relationship, no repeat experiment.				
Micronuclei in BM cells of mice (4 an./dose) ip expo., single sacrifice : 30 60 h duration 1000 PCE/animal analysed C- : solvent : DMSO	25, 50 100 mg/kg	Cuman L **(trade name)	**positive** (dose dep. time dep.)	Hemavathi Rahiman, 1993
Comment: the compound is of unknown purity. Recommended sampling times are 24 and 48 h.				

* statistically significant

** Cuman L contains 27% active substance and 73 % inert ingredients.

Table B.5.4.4-6 : Tests for mitotic recombination

experimental conditions	**Doses**	**Product purity**	**Results**	**Reference**
Wing mosaic assays in *D. melanogaster* treat. time : 48 h, 72 h 96 h	0, 1.3 x 10^{-2} - 7.8 x 10^{-2}	Cuman L *(trade name)	- freq. small single spots with mhw phenotype, - freq. large single spots with duration of exposure	Tripathy et al., 1989
Female germ-line mosaic assays in *D. melanogaster* treat. time : 48 h, 72 h 96 h	0, 1.3 x 10^{-2} - 7.8 x 10^{-2}	Cuman L* (trade name)	- % mosaic induction	Tripathy et al., 1989
Eye mosaic assays in *D. melanogaster* treat. time : 48 h, 72 h 96 h	0, 1.3 x 10^{-2} - 7.8 x 10^{-2}	Cuman L* (trade name)	- % mosaic spots (twin light spots)	Tripathy et al., 1989

*Cuman L contains 27% active substance and 73% inert substance.
The purity of the compound is unknown in the 3 assays .

3. Other indicators of genetic damage:

Morphological sperm abnormalities :

Table B.5.4.4-7 : Morphological sperm abnormalities

Tissue, experimental conditions	**Doses**	**Product, purity**	**Results**	**Reference**
Sperm shape abnormalities in mice Swiss Albino male mice (6 an./group) ip expo., single or repeated 5 days sacrifice 1 month after treat. C- : solvent : DMSO	single : 50 100 mg/kg repeated : 25 mg/kg	Cuman L (trade name)	**positive** : freq. abnormal sperm (acrosome, head tail morphology): 5.65 % at 50 mg/kg 8.18 % at 100 mg/kg (C: 1.31 %) 8.37 % at 25 mg/kg (repeated)	Hemavathi Rahiman, 1993

No data on positive controls for demonstrating the sensitivity and thevalidity of the test.

B.5.4.5 Summary of genotoxicity of Ziram (Annex IIA 5.4):

Ziram, after metabolic activation, induced base-pair substitution in strain TA100 of *S. typhimurium*. A gene mutation test in mammalian cells was not supplied. This point has to be addressed by the notifiers . Ziram did not induce chromosome aberrations in CHO cells *in vitro*, and in mouse bone marrow cells and spermatogonia *in vivo*. It did not induce micronuclei in peripheral erythrocytes after sub-chronic exposure *in vivo*. In another study, ziram did not induce micronuclei in mice bone marrow after oral exposure. Ziram did not induce DNA repair activity in hepatocytes in culture.

Table B.5.4.4-1 : Summary of genotoxicity of Ziram

Type of test	Result	Product, purity , batch number	References
***In vitro* genotoxicity tests:** Ames test TA1535, TA1537, TA1538,TA98 and TA100+/- S9	positive	98.5%; B.n°8331 AA	Jones et al., 1990 task force study
Gene mutation in mammalian cells	no data	-	-
Chromosome aberrations in CHO cells, strain K1-BH4	negative	98.5%; B.n°8331 AA	Brooker and Akhurst, 1989 task force study
UDS in rat hepatocytes	negative	98.5%; B.n°8331 AA	Proudlock, 1989 task force study
***In vivo* genotoxicity tests:** Micronucleus assay in mice peripheral blood (89 day, oral)	negative results are accepted if further information on toxicity are provided	98.8%; 8331AA	Proudlock and Taylor, 1992 task force study
Micronucleus assay in mice bone marrow	negative	97.4%;b.n° 4/52019	Ciliutti and Brightwell, 1995, Bakelite
Mouse bone-marrow chromosome aberration test (oral adm.)	negative	98.5%; B.n°8331 AA	Völkner, 1992a task force study
Chromosome aberration in mouse spermatogonia	negative	98.4%; b.n°.8331 AA/V528	Völkner, 1992b task force study
UDS *in vivo* using rat hepatocytes	negative	97.4%;b.n° 4/52019	Falezza and Brightwell, 1995, Bakelite

Conclusion from the data in the open literature:
Positive tests in *S.typhimurium* and in mammalian cells *in vitro* suggest that ziram has to be regarded as a substance with mutagenic potential in pro- and eukaryotic cell systems *in vitro*.
However, very often, data were reported insufficiently so that a quantitative evaluation of the results is impossible. Nearly all the studies were not realized according to current qualitative standards and guidelines.
In vivo studies positive results were reported for the ability of ziram to induce gene mutations in *Drosophila melanogaster*. Despite the strengths of Drosophila assays, they do not provide sufficient basis for predicting human risks. Although metabolism and gametogenesis in *Drosophila* are analogous to the mammalian processes, they are not identical. Means of exposure and the measurement of dosages in flies also differ from mammalian toxicology. *In vivo* studies did not add significantly to the evaluation of ziram *in vivo* genotoxicity , because of pitfalls in the experimental design of the studies and/or unclear relevance of the endpoint analysis.
The mechanism leading to spermhead anomaly and its genetic meaning is not clear so far. With regard to a mutagenic potential of ziram to be investigated the sperm anomaly test is irrelevant.

On the basis of the data undertaken by the notifier with high grade a.s., according to current qualitative standards and guidelines, ziram gave negative results in the *in vivo* tests and it is therefore believed that the classification of ziram as an *in vivo* established mutagen is questionable.

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

B.5.5.1 Long-term (2 years) oral toxicity in the rat (Annex IIA 5.5)

- Rats, 104 weeks, 60, 180, 540 ppm (Powell et al., 1994a, Task force study)

Findings :
Mortality was not affected.
Statistically significant effects in haematological parameters were observed in both male and female rats receiving 180 or 540 ppm. The small intergroup differences seen for these dosage levels were considered not to be of toxicological significance.
Minor but consistent changes in biochemical parameters occurred in both males and females receiving 180 or 540 ppm. At 60 ppm, values were within the expected ranges and the small intergroup differences seen for this dosage group were considered not to be of toxicological significance.
Effects on the urinalysis parameters measured were seen at 180 or 540 ppm but not considered to be associated with any histopathological changes in the kidney.

Table B.5.5.1-1 : Long-term (104 weeks) oral toxicity of ziram in the rat

Endpoints/dose	0 ppm		60 ppm		180 ppm		540 ppm	
	_	_	_	_	_	_	_	_
Mortality (%)								
1-104 week	36	68	44	56	54	50	38	52
Mortality (%)								
1-52 week	10	0	5	5	5	5	15	0
Clinical signs			no particular effect related to ziram					
Body weight			î(1.6%)	(î0.9%)	(î7.3%)	(î1.0%)	(î11%)	(î17%)
Body weight gain							î14%	î26%
Overall food intake							î9 %	î 6%
Ophtalmoscopy			no lesions indicative of reaction to treatment					
Haematology:								
PCV								î
Hb								î
RBC						î		î
MCHC							ì	
MCV							î	ì
Clinical chemistry								
AP							ì(42%)	
bilirubine								ì(50%)
Urinalysis:protein								î
Organ weight(r)								
testes+epididimis					ì12%		ì26%	
adrenals							î34%	ì56%
liver								ì12%
brain								ì19%
thyroid								ì33%
heart								ì11%

Statistically significantly different from control : $p \leq 0.05$ (Kruskal-Wallis analysis ( ) : not statistically significantly different.
(r): relative weight

Macroscopic pathology:
The table B.5.5.1-2 summarizes all suspected treatment-related findings, and major post-mortem findings of the remaining organs and tissues.

No corpora lutea were visible in the ovaries of females and microscopic examination revealed no toxicologically significant changes. Depressions and thickening of the forestomach were seen in both sexes with raised areas noted in males and white discolouration noted in females. Depressions were found in the stomach antrum mucosa in females only various cystic changes were noted in the adrenals of females.

Table B.5.5.1-2 : Macroscopic pathology in 50 animals (decedents + terminal)

Endpoints/dose	**0 ppm**		**60 ppm**		**180 ppm**		**540 ppm**	
	_	_	_	_	_	_	_	_
Forestomach:								
depressions	11	8	16	7	20	14	20	31
raised areas	0	0	0	1	3	1	4	0
thickening	5	3	11	3	11	8	12	12
white discolouration	4	1	8	1	7	4	6	9
Stomach antrum mucosa depression	6	2	4	3	6	6	4	8
adrenals cysts	0	0	0	0	0	1	0	5
cystic enlargement	0	0	0	0	0	0	0	2
Skeletal muscle:								
atrophied hindlimbs	4	0	5	0	3	0	6	2
ovaries:								

Micropathology of animals dying during the study or killed at termination:

- Neoplastic changes:

Benign haemangiomata were seen in the mesenteric lymph nodes (draining the intestinal tract) of five male terminal kill rats receiving 540 ppm ziram. This incidence was statistically significant in comparison to the control group by Fisher's test. Haemangiomata were not observed in other tissues of these animals and were not seen in any rats receiving lower doses of ziram. Haemangiomata are benign multilocular tumours occasionally seen in low numbers of rats of this strain and age range, mesenteric lymph nodes and the spleen being the commonest sites for their detection. The incidence which was recorded in the 540 ppm dosage group of this study of some 5/50 animals in a single site (6/50 in total) is outside the normal background incidence for this laboratory where a range from 0/50 in the majority of instances to a maximum of 2/50 in control animals is recorded for a series of recent and concurrent studies utilising this strain of rats .

Statistical analysis of the relative group incidence for this tumour type by the time-to-tumour methods recommended by the IARC demonstrated a statistically significant dose-related trend in the number of animals with tumours (P=0.0001) and a significant difference between the control and the 540 ppm dosage group (P=0.024).

It is therefore, considered that the presence of these haemangiomata at a dosage level of 540 ppm in male animals was a consequence of the administration of ziram.

Table B.5.5.1-3 : Micropathology in 50 animals (decedents + terminal)

Endpoints/dose	**0 ppm**		**60 ppm**		**180 ppm**		**540 ppm**	
	_	_	_	_	_	_	_	_
Neoplastic changes								
Spleen: haemangioma	0	0	0	0	0	0	1	0
Mesenteric lymph nodes: hemangioma(benign)	0	0	0	0	0	0	5*	0
Non-neoplastic changes								
Spleen: haemosiderosis	10	24	22*	29	29*	38*	23*	39*
Skeletal muscle : adipose infiltration /replacement of peripheral muscle fibre bundle	5	0	10	4	27*	21*	43*	26*
narrow peripheral muscle fiber	2	0	10*	4	30*	15*	37*	22*
Sciatic nerve: axonal degeneration	14	3	12	5	15	4	22	16*

Endpoints/dose	0 ppm		60 ppm		180 ppm		540 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver: bile duct hyperplasia	7	5	7	6	13	9	15	17*
pigmented sinusoidal cells	2	0	5	3	22*	13*	26*	15*
Kidneys: cortical tubular epithel.cells: brown granular pigment	2	5	6	8	6	9	4	19*
Pancreas: replacement by adipose tissue	7	0	14	2	15*	4	21*	3
Adrenals vacuolation	4	1	11*	1	11*	0	12*	2
cortical cystic degenerescence	2	7	3	9	1	12	2	29*
Thyroid: prominent ultimobranchial cyst	4	3	5	12*	14*	22*	15*	27*
C-cell hyperplasia	1	5	3	5	5	5	8*	3
Stomach:hyperplasia non glandular	6	7	18*	6	25*	15*	25*	37*
ulceration or oedema non glandular	5	3	8	5	14*	6	14*	12*
Parathyroids: hyperplasia	1	1	4	0	4	1	7*	0
Seminal vesicle: depressed secretion	2	-	5	-	6*	-	5	-
*statistically significantly different from control - Fisher's exact test								

- Non-neoplastic changes:

Skeletal muscle: an increase in the incidence of adipose replacement and narrowing of myofibres in peripheral muscle bundles was recorded in male and female rats receiving 180 and 540 ppm of ziram. Some increase in the incidence of narrowed myofibres was also noted in male rats receiving 60 ppm. At this dose level, similar changes were observed in a small number of females. Although they were absent in female controls, the incidence at 60 ppm was comparable to the control incidence for males and it is unlikely that a treatment-related effect was present for females at this low dose level.

The increase in *sciatic nerve* lesions is probably associated with the increase in skeletal muscle changes. Minor degree of axonal degeneration comparable to that seen as part of the background pathology of ageing in control animals was noted in the sciatic nerves of rats from the low and intermediate dose groups.
In the spinal cords of animals at all dosage levels some degree of minor axonal degeneration was noted but there was no clear dose relationship in degree or incidence and it was not considered that a treatment effect was involved.

Spleen:
An increased incidence and amount of Perls'positive brown pigment aggregation (haemosiderosis) was seen in the spleen of both sexes at 540 ppm and in males receiving 180 and 60 ppm. Hemosiderin is a pigment, which probably consists of ferritin aggregates. Ferritin is a iron-binding protein that functions as an iron depot in the body under normal physiological conditions. If there is an excessive supply of iron, for example in cases of extreme hemolysis, iron is also stored in the form of haemosiderin. This pigment is a common background finding in a proportion of ageing animals (see controls) but is exacerbated by treatment with ziram.

In the *liver*, the pigment deposition seems likely to be linked to the reduced PVC, Hb and RBC noted haematologically in these groups and may be attributable to an increased rate of erythrocyte destruction. The increased liver weights reported at the terminal kill may be associated with this observation and also with the bile duct hyperplasia statistically increased in the high dose group. In almost all cases the degree of hyperplasia was minimal to moderate in both control and treated rats.

Kidneys : the increased incidence of brown pigment is considered to be related to treatment with ziram but is unlikely associated with the urinalysis effect reported previously.

Pancreas: an increased incidence and severity of adipose tissue replacement of exocrine pancreas was noted in male rats at 180 and 540 ppm. At 60 ppm, a similar increase in male rats did not quite achieve statistical significance. There was no concomitant treatment-related effect seen in respect of exocrine atrophy.

Hypertrophic and degenerative lesions of the *adrenal cortex* are a normal occurrence in ageing rats of this strain; this effect was exacerbated and may explain the reduction in adrenal weight noted.

Thyroid : an ultimobronchial cyst is a developmental anomaly occasionally seen in a proportion of normal rat thyroid gland and represents the remnants of the embryonic ultimobranchial body, responsible for the distribution of calcitonin secreting C-cells in the thyroid. A small increase in C-cell hyperplasia was seen in occasional male rats (primarily at termination) at the 540 ppm dosage level but was not detected in females and thus the aethiology of this change remains equivocal.

Stomach : increased incidence and severity of hyperplastic and/or ulcerative lesions of the nonglandular stomachepithelium was more pronounced in male than in female animals and are in accordance with the macroscopic observations for these tissue.
The *gastric epithelial* changes are thought to be the consequence of the irritant action of the compound.

Table B.5.5.1-3 : Main factors contributory to death

Endpoints	**Doses (ppm)**							
	0		**60**		**180**		**540**	
	–	–	–	–	–	–	–	–
Examined	19	34	22	28	27	25	19	26
Pituitary adenoma	5	22	5	14	9	13	6	19
Pituitary adenocarcinoma	-	3	-	5	-	3	-	2
Multiple mammary tumour	0	7	1	8	0	4	0	6
subcutaneous tumour	3	3	6	1	3	0	3	3
Lymphoreticular tumour	0	1	4	0	2	2	1	0
Fibrosarcoma	1	0	0	0	0	1	0	1
Leiomyosarcoma	1	0	0	0	0	2	0	0
Squamous cell carcinoma		0		0		0		0
Intraabdominal haemorrhage	0	0	1	0	0	0	0	1
gastric ulceration	0	0	1	1	0	0	2	2
carcinosarcoma	0	-	0	-	1	-	0	-
Atrophic skeletal muscle	2	-	0	-	2	-	1	-

All other changes were within the normal background range of incidence for rats of this strain and age and were not considered to be of any toxicological significance. No histological changes were reported that might explain the increased heart weights in female animals of the high and intermediate dosage groups, nor the decreased pituitary weights in high dose group male animals.
No treatment-related changes were noted in the group distribution of those factors considered to be contributory to the deaths of those animals killed or dying prior to the termination of the study (Table B.5.5.1-3)

Conclusion : Treatment-related changes from the 60 ppm group on, were degenerative/atrophic changes of the skeletal muscle (males), haemosiderin in the spleen (males), adipose replacement of exocrine pancreas (males), cortical degeneration of adrenals (males), prominent ultimobranchial cysts in the thyroid (females) and hyperplastic and erosive lesions of the stomach (males) . The lowest dose of 60 ppm cannot, therefore, be considered as a NOAEL.
The tumorigenic effect in male animals receiving 540 ppm ziram was limited to haemangiomata and there was no evidence of malignancy.

Guidelines:
Experimental protocol not fully in compliance with the test method B.30 of Directive 87/302/EEC or OECD 452 (1981)
Deviation from the official protocol: GGT was not measured; this study is a combined toxicity and carcinogenicity study and 4 doses are, therefore, preferable.
GLP status : yes, but no attest of competent authority.
Material and methods :
50 Crl:CD(SD)BR rats/sex/dose received in the diet , ziram (technical; 98.7%; b.n°.8331A) at dosage levels of 60, 180 or 540 ppm for 104 weeks.
A further three groups of 20 rats/sex (satellite groups) received the same dosage for 52 weeks.
Converted doses in males week 1-104: 2.5; 7.7 or 23.7 mg/kg bw/day.

females: 3.4; 10.2 or 34.6 mg/kg bw/day.
Converted doses in males week 1-52: 3; 9.1 or 27.3 mg/kg bw/day.
females: 3.9; 11.7 or 37.5 mg/kg bw/day.

- Data from open literature:

- Rat, dietary administration for 24 month at 20, 200 or 2000 ppm corresponding to 1, 10 or 100 mg/kg bw/d (Enomoto et al., 1989) and (Maita et al., 1997)

Findings :
In this study, the top dose was 4 times higher than in the task force study .
Clinical signs: 3 male rats had shown ataxic gait with immobility of the hind-limb several weeks before termination of the study.
At 2000 ppm, retarded growth together with decreased food intake. A trend of slight anemia manifested by decreases in hematocrit, hemoglobin or erythrocyte count was persistently observed , at the top dose for both sexes. Anemia was a specific toxic response to rats given ziram. Elevation of ALT and cholesterol might suggest that ziram gave toxic effects to the liver . In accordance with these blood parameters, significantly higher values of relative weights of the liver were recorded at the top dose providing an additional evidence of liver toxicity by ziram treatment.
At the top dose, male and female rats showed enlargement of the thyroid associated with follicular hypertrophy, a goitrogenic effect. Moreover, in this study, the incidence of C-cell hyperplasia, which is regarded as a preneoplastic lesion of C-cell tumor, was significantly depressed in males of the 2000 ppm group. Oposite effects were observed in the NPT study and in the Task Force report.
At 2000 ppm, epiphyseal abnormalities in the long bones of the hind legs were observed in both sexes. While females had neither clinical signs nor gross abnormalities during the study, histopathological examination revealed retarded epiphyseal closure of the proximal end of the tibia in both sexes. Females also showed the epiphyseal lesion at the distal end of the femur. In severely affected rats marked proliferation of epiphyseal cartilaginous tissue was also noted together with the irregular arrangement of chondrocytes. These changes were evident only in aged animals. The occurrence of the lesions appeared to be caused by impaired regulation of epiphyseal closure which might be related to the treatment with ziram. Epiphyseal closure is influenced by various factors including hormones, vitamins and nutrition. The treatment with ziram might have affected some of these factors since treated rats in the top dose group showed depressed bw gain with low food consumption, decreased values of erythrocyte indices, low serum calcium level, and thyroid hypertrophy indicating the presence of hypothyroidism. Atrophic or regressive changes were recorded simultaneously in the sciatic nerve, calf muscle(*M.triceps surae*) and crus bone (tibia).
Among the neoplastic lesions, the incidence of interstitial tumor was sporadically high in males of the 2000 ppm group. Their incidences were within the ranges of historical data (table B.5.5.1-4).

Table B.5.5.1-4 : 104 week study of ziram in rats

Endpoints	**Dose (ppm)**							
	0		**20**		**200**		**2000**	
	_	_	_	_	_	_	_	_
bodyweight				î			î19%	î27%
food consumption							î10%	î18%
hematocrit							î	î
hemoglobin							î	î
erythrocyte count							î	î
Ca^{++}							î	î
Total cholesterol							ì	ì
ALT					ì	ì		
Organ weight (a/r)								
thyroid								ì/ì
liver							ì/ì	ì/ì
kidneys							î/	ì/
spleen							/ì	
Calf muscle							î/î	î/î

Endpoints	Dose (ppm)							
	0		20		200		2000	
	_	_	_	_	_	_	_	_
Microscopic lesions:								
n° animals examined	80	80	79	80	79	79	80	79
Bone marrow ì hematopoieisis	10	7	10	4	4	3	21*	23*
Spleen ì extramedullary hematopoieisis	11	13	9	16	9	13	8	33*
Forestomach hyperkeratosis	5	1	1	6	13*	11*	46*	34*
thyroid follicular hypertrophy	2	1	4	1	3	9*	64*	65*
C-cell hyperplasia	11	8	6	5	6	4	2*	3
Sciatic nerve atrophy	0	0	0	2	0	0	30*	26*
Calf muscle atrophy	1	2	4	0	49*	25*	68*	46*
Retarded closure of epiphyseal plate in tibia	0	1	0	0	0	5	22*	13*
Tumors								
interstitial cell tumors testis	51		54		55		64*	
Thyroid								
follicular adenoma/adenocarcinoma	1/1	0/0	0/0	1/1	0/0	0/0	2/1	0/0
C-cell adenoma/carcinoma	11/0	6/0	11/1	6/1	15/3	6/2	7/1	1/1

* Statistically significantly different from control

Conclusion :
It remains difficult to identify the factors responsible for the epiphyseal lesions , this kind of changes is commonly observed in toxicity studies with chemicals. The lesions only appeared in the later stages of the experiment and proliferation of epiphyseal chondrocytes was observed in aged animals . The lesions could not be ascribed to either directly stimulated chondrogenesis or disturbed ossification of epiphyseal cartilage. They might be related to ziram-induced hypocalcemia because of an increased gastric calcium secretion caused by zinc. Ziram, however, has been shown to produce thyroid C-cell adenoma (see NTP/NIEHS, 1983), potentially increasing calcitonin production, which, on the contrary, would produce hypocalcemia by retaining calcium in the bone and decreasing bone resorption. The effects on the thyroid became critical in the assessment of ziram toxicity and further explanation will be needed to clear if ziram treatment may increase or decrease the proliferating lesions of C-cells in thyroid.
The NOAEL in this study = 20 ppm = 0.7 mg/kg bw/d.

Material and methods : 80 F344 rats /sed/dose received ziram (97.5% ; Ouchi-shinko chem.Ind.Co.Ltd) at 0, 20, 200 or 2000 ppm for a period of 104 weeks. Converted dose : for males , 0, 0.70, 6.9 and 74 mg/kg bw/d, and for females 0, 0.83, 8.5, and 91 mg/kg bw/d.

- Rats, 103 weeks, diet, 300, 600 ppm (NTP/NIEHS, 1983)

In this study, the same range of doses were used as in the task force study. However, in this study, ziram contained 6.5% thiram.

Findings :
Survival , food consumption and mean body weights of rats were not affected. C-Cell carcinomas of the thyroid in male rats occurred with a statistically significant positive trend and the incidence in the high dose group was significantly higher than that in controls and higher than that previously observed in control male rats at the same laboratory (18/584, 3%; range 0% to 8%). The combined incidence of males with either C-cell adenoma or carcinoma also showed a statistically significant positive trend . There were no significant histopathologic changes noted in the follicular cells(Table B.5.5.1-5).

Table B.53.5.1-5: tumor incidence in rats exposed to ziram.

Primary tumors	0		300 ppm		600 ppm	
Overall tumor rate	_	_	_	_	_	_
Thyroid: follicular -cell adenoma or carcinoma	1/50	-	5/49	-	1/49	-
Thyroid: C-cell carcinoma	0/50		2/49=4%		7/49=14% ì*	
Thyroid: C-cell adenoma or carcinoma	4/50 =8%	9/50	9/49 =18%	9/50	12/49 *ì =24%	9/50

Statistically significant according to the Cochran-Armitage trend test (ì = p<0.05) ; Fisher Exact test * = p<0.05.

Comment and conclusion :
Thyroid C-cell tumours are common, spontaneous tumours in rats as illustrated in table Table B.5.5.1-6.
Table B.5.5.1-6 : Comparative thyroid C-cell tumours % incidence in different rat strains (Gopinath, 1997)

Strains	Sprague-Dawley		Fischer 344		Wistar (Han)	
	Male	Female	Male	Female	Male	Female
adenoma	6	4	12	9	5	3
carcinoma	3	3	4	3	2	1

In the above mentioned, ziram task force study, a small but statistically significant increase in C-cell hyperplasia was reported in males of Sprague-Dawley strain at 24 mg/kg bw/d. In the study of Maita et al(1997), the incidence of C-cell hyperplasia was significantly depressed in F344 rats. In this NTP/NIEHS study, ziram appears to increase the incidence of C-cell adenoma and/or carcinoma in male rats already at the dose of 11 mg/kg bw/day. In this study, however, ziram was adulterated with thiram (for 6.5%) which is known to produce an increased incidence of C-cell hyperplasia at a dose of 9 mg/kg bw/day (2 year study). It could be argued that the combination of ziram and thiram would produce a synergistic effect, although it remains quite difficult to conclude that from three totally different experiments.

Ziram seems to be carcinogenic in male rats, causing an increased incidence of C-cell hyperplasia, adenoma and carcinoma.

Material and methods:
50 F344/N rats/sex received in the diet ziram (89% with 6.5% thiram) for 103 weeks which corresponds to an average daily consumption of about 11 and 22 mg/kg bw /d for males and 13 and 26 mg/kg bw/day for females.

B.5.5.2 Carcinogenicity study in the rat (Annex IIA 5.5)

see point B.5.5.1

B.5.5.3 Carcinogenicity study in the mouse (Annex IIA 5.5)

- Mouse, dietary administration for 80 weeks at 25, 75, 225, or 675 ppm (Powell et al., 1994b, Task force study)

Findings :
Mortality : 130 deaths during the 80 weeks of treatment . There was no indication of a treatment-related effect on the incidence or distribution of decedents.

Organ weights : changes such as decrease in absolute or relative brain weight (from 75 ppm), increase of relative testes and epididymus weight (from 225 ppm) and increased kidney weight (at 675 ppm) were considered not to be of toxicological significance and are an effect of the lower mean body weight of these groups.

Macroscopic changes : adipose tissue reduction noted in 11/17 decedents at 675 ppm, roughened (6/50 at 675 ppm) or discolorated (10/50 at 675 ppm) forestomach, irregular kidneys cortical scarring (14/33 at 675 ppm) or brown kidneys(5/50 at 675 ppm) were not related to any histopathological findings and therefore were considered not to be of toxicological significance.

Microscopic pathology:

- Neoplastic lesions did not reveal any treatment-related effect, either on the incidence of any tumour type or on the total number of mice with benign or malignant neoplasms per group. The incidence of the various neoplasms observed in this study showed no significant deviation from the expected tumour profile of laboratory-maintained mice of this strain. In this study lymphoid tumours and pulmonary tumours among male and female mice and liver tumours among male mice were among the most frequently observed tumours. Other type of tumours were of low incidence (table B.5.5.3-1).

Table B.5.5.3-1: Neoplastic morphology incidence in surviving or decedent mice

Endpoint/ dose	**0**		**25 ppm**		**75 ppm**		**225 ppm**		**675 ppm**	
sex	_	_	_	_	_	_	_	_	_	_
D/S	16D/34 S	12D/38 S	16D/34 S	15D/35 S	9D/41S	10D/40 S	12D/38 S	10D/39 S	17D/33 S	15D/35 S
Lung adenoma (B)	1D/8S	6S	1D/6S	1D/5S	11S	3D/6S	1D/7S	1D/6S	2D/7S	2D/6S
carcinoma (M)	3D/2S	1S	1D	0	1S	1D/1S	0	1S/1S	1D/3S	2S
lymphoma (M)	0	3D/2S	0	2D/1S	1D/1S	2S	1D/1S	5D/2S	2D	1S
Liver adenoma (B)	1D/3S	0	1D/5S	0	1D/6S	0	2S	0	2S	0
carcinoma (M)	1D/4S	0	1S	0	3S	0	2S	0	0	0
hemangioma (B)			1S							
Statistical ananlysis not performed ; (M) = malignant; (B) = benign										

- Non-neoplastic findings and treatment-related findings:
An increased incidence of centrilobular enlargement, sometimes with vacuolation, and/or generalised enlargement of hepatocytes was seen in males and females from all treated groups when compared to controls. There was no clear dose-relationship. The severity of this change was only minimal in most of the cases and this change was not associated with liver weight changes of macroscopic observations. These changes were not associated with any changes in the liver weight or macroscopic abnormalities. These types of changes are often observed in long term rodent studies using various unrelated xenobiotics and the nature of the change is an indication of an adaptative response.
An increased incidence of epithelial hyperplasia in urinary bladder was seen in male and female mice receiving 675 ppm and in males receiving 225 ppm when compared to control mice. The severity of this change was only minimal in most of the cases reported (table B.5.5.3-2).

Table B.5.5.3-2 : Histopathology in some mouse tissues

Dose/endpoint	**0**		**25 ppm**		**75 ppm**		**225 ppm**		**675 ppm**	
	–	–	–	–	–	–	–	–	–	–
Liver/number examined	50	50	50	50	50	50	50	50	50	50
centrilobular hepatocyte enlargement	2	0	21*	12*	21*	19*	25*	16*	19*	13*
centrilobular hepatocyte enlargement and vacuolation	0	0	7*	0	9*	2	5*	1	5*	1
generalised enlargement	1	1	9*	10*	12*	10*	15*	7*	6	7*
Urinary bladder/number examined	50	48	50	50	46	48	50	49	50	49
epithelial hyperplasia	7	0	7	5*	9	1	20*	5*	31*	14*

* Statistically significantly different from control (Fisher's exact test)

Conclusion :
Ziram did not induce tumours in mice at dose levels up to 675 ppm during 80 weeks.
In all treated groups, increased incidence of centrilobular and/or generalised hepatocellular enlargement was seen.
Urinary bladder epithelial hyperplasia was observed in males and females receiving 675 ppm and in males receiving 225 ppm.
The lowest dose induced centrilobular enlargement, sometimes with vacuolation, and/or generalised enlargement of hepatocytes. No NOAEL was defined in this study.

Guidelines:
Experimental protocol not fully in compliance with method B of Directive 87/302/EEC or OECD guideline 451(1981).
Deviation from official protocol: blood examination was not performed in all exposed animals.
GLP status : yes (no attest of competent authority).
Material and methods :
50 mice (Crl:CD-1(ICR)BR) /sex/dose were given Ziram (98.7%; b.n°.8331 AA) in the diet at 25, 75, 225 or 675 ppm for at least 80 weeks. Diet was prepared weeky.
Achived intake: 3, 9, 27, 82 mg/kg bw/d for males
and 4, 11, 33, and 95 mg/kg bw /day for females.

- Mice, 103 weeks, diet, 600, 1200 ppm (NTP/NIEHS, 1983)

Findings :
Table B.5.5.3-3: Pathology and statistical analyses of results.

Endpoints	Dose (ppm)						
	0		600		1200		statistics
	_	_	_	_	_	_	
lung alveolar/bronchiolar adenoma	6/49 12%	2/50 4%	5/50 10%	5/49 12%	8/49 20.4%	10/50* 20%	_: significant dose-response trend
lung alveolar/bronchiolar adenoma or carcinoma	8/49 16%	4/50 8%	8/50 16%	6/49 12%	12/49 24%	11/50 22%	_: significant incidental tumor trend test
liver adenoma	6/49 12%	7/50 14%	1/50 2%	2/50 4%	8/49 16%	0/50* 0%	
liver adenoma or carcinoma	19/49 39%	9/50 18%	9/50 18%	4/50 8%	9/49 18%	1/50 2%	
hepatocellular carcinoma	13/49 27%		8/50 16%		1/49* 2%		
mammary fibroadenoma		16/50 32%		17/50 34%		8/50 16%	
pulmonary adenomatous hyperplasia	15/49 31%	18/50 36%	19/50 38%	27/49 55%	16/49 33%	26/50 52%	

* statistically significantly different from control

Survival of mice was not affected. Mean body weight gain was depressed in male mice throughout the study and by high dosed females after 80 week. Average daily food consumption was reduced. The incidence of alveolar/bronchiolar adenomas was significantly ($p<0.05$) increased in female mice .
The combined incidence of alveolar/bronchiolar adenomas or carcinomas in female mice showed a statistically significant positive trend ($p<0.05$). The incidence in the high dose group was significantly ($p<0.05$) higher than that in the controls.
Pulmonary adenomatous hyperplasia consistent with chronic Sendai virus infection (confirmed by serologic analysis performed on untreated animals from the same animal shipment and present in the same room) was observed in control and dosed male mice as well as in control and dosed female mice .
Six of the 26 high dosed females with pulmonary adenomatous hyperplasia also had pulmonary tumors, whereas 4 of the 24 high-dose females without pulmonary adenomatous hyperplasia also had pulmonary tumours. Only 1 of 27 low dose females with adenomatous hyperplasia had a pulmonary tumour.
There was a significant decrease in mammary fibroadenomas in top dose females.
Significant dose-related decreased incidences of liver carcinomas in male mice and of liver adenomas in female mice (table B.5.5.3-3).

Conclusion : It remains difficult to interpretate the increased incidence of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas in female mice because of the simultaneous presence of a Sendai virus infection. Ziram was not carcinogenic for male B6C3F1 mice.

Material and methods:
50 B6C3F1 mice/sex received in the diet ziram (89% with 6.5% thiram) for 103 weeks which corresponds to an average daily consumption of about 122 and 196 mg/kg bw /d for males and 131 and 248 mg/kg bw/day for females.

B.5.5.4 Mechanism of action and supporting data (Annex IIA 5.5)

No data (not necessary).

B.5.5.5 Summary of long- term oral toxicity (Annex IIA 5.5)

As in the short-term repeated dose experiments, long-term administration of ziram in rats produced decreases in body weight gain, food intake, and erythrocyte parameters suggestive of anemia.

Furthermore, rats showed the following treatment-related tissue changes: haemosiderin deposits in spleen and liver, cortical degeneration of adrenals (males), increased prominence of ultimobranchial cysts in the thyroid and C-cell hyperplasia (females), degenerative/atrophic skeletal muscles (males), axonal degeneration in spinal cord and sciatic nerves, adipose replacement of exocrine pancreas (males), lipofuschin accumulation in kidneys (females), bile duct hyperplasia in the liver, and hyperplastic and erosive lesions of the nonglandular epithelium in the stomach (males). Some of these changes already appeared at the lowest dose studied, 2.5 mg/kg bw/day and, therefore, the identification of a NO(A)EL was not possible.

At 24 mg/kg bw/d, male rats showed treatment-related haemangiomata in mesenteric lymph nodes and spleen. These haemangiomata were not pronounced, did not show evidence of malignancy and were confined to this group and sex only.

Studies from the open literature confirmed the ziram-induced muscular atrophy and sciatic nerve degeneration. Moreover, at doses higher than in the controlled studies, ziram was found to produce skeletal lesions and thyroid hypertyrophy. In these studies the NOAEL was 0.7 mg/kg bw/d. In contrast to the well controlled study, one of the published studies showed that ziram, containing 6.5% of thiram known to produce C-cell hyperplasia and adenomas in female rats at the dose of 8 mg/kg bw/d, induced thyroid C-cell adenomas and carcinomas in male rats, statistically significant at a dose of 22 mg/kg bw/d. This observation might form a continuum with the C-cell hyperplasia seen in the well controlled study. In a similar study conducted on F344 rats, the incidence of C-cell hyperplasia was significantly depressed in males at 74mg/kg bw/d. In this study, male and female rats, at 74mg/kg bw/d, showed enlargement of the thyroid associated with follicular hypertrophy.
The effects on the thyroid became critical in the assessment of ziram toxicity and further explanations will be needed to clear if ziram treatment may increase or decrease the proliferating lesions of C-cell in the thyroid.

In mice, ziram did not induce tumours at dose levels up to 82 mg/kg bw for 80 weeks. In all treated groups, increased incidences of centrilobular and/or generalised hepatocellular enlargement were seen. Urinary bladder epithelial hyperplasia was observed in males and females receiving 82 mg/kg bw and in males receiving 27 mg/kg bw. The NOAEL was lower than 3 mg/kg bw/d. Pulmonary tumours, found in one study, occurred in the presence of a virus infection which makes any interpretation difficult.

On the basis of the increased incidence of haemangiomata and thyroid C-cell hyperplasia, adenomata and carcinomata, a tumorigenic effect of high doses of ziram in the rat cannot be excluded. Since the overall conclusion with regard to mutagenicity is negative, the possible tumorigenic mechanism remains unclear.

Table B.5.5.5-1 : Summary of long-term toxicity of Ziram

study	**NOAEL** mg/kg bw/d	**LOAEL** mg/kg bw/d	**critical effects**	**purity (%) batch number**	**references**
rat, 104 wk, oral	< 2.5	2.5	skeletal muscle degenerative/atrophic changes, haemosiderin in spleen, adipose replacement of exocrine pancreas, cortical degeneration of adrenals, prominent ultimobranchial cysts in the thyroid, hyperplastic and erosive lesions of the stomach. (hemangioma (benign) at 23.7 mg/kg bw/d in male rats)	98.7%; b.n°.8331A	Powell et al., 1994a, Task force study
mouse, oral, 80 weeks	< 3	3	centrilobular enlargement, sometimes with vacuolation of hepatocytes reduced food consumption	98.7%; b.n°.8331AA	Powell et al., 1994b, task force study

B.5.6 Reproductive Toxicity (Annex IIA 5.6)

B.5.6.1.1. Two generation reproductive toxicity in the rat (Annex IIA 5.6.1.)

- Rat exposed to 0, 280, 1260 or 2800 ppm ziram into the diet (Ritter and Bowie, 1982, task force study)

Guidelines:
Experimental protocol not in compliance with test method B.5 of Directive 87/302/EEC.
Deviation from the official protocol: no enough pregnant females. Dead parents were not necropsied. Food consumption measurements and body weight were not performed at the recommended periods.The report is rough and sometimes unreadable with errors in tables, schemes etc... !
GLP status : no, GLP were not in existence at the time the study was performed.
Material and methods :
10 male and 20 female rats per dose group were initially assigned dose levels of 0, 280, 1260 or 2800 ppm ziram into the diet. These levels produced unacceptable toxicity to the parents as well as to the offsprings and the levels were reduced on day 119 to 0, 140, 770 or 1400 ppm respectively. Mating was initiated at day 75 after dosing was begun. The study is not accepted.

- Rat, 60, 180 (fortified to 72 and 207 ppm respectively due to the instability in the diet) and 540 ppm. (Nemec, 1996, task force study)

Findings:

Table B.5.6.1.1-1: Two generation reproductive toxicity in the rat

dose/endpoint	**0 ppm**		**72 ppm**		**207 ppm**		**540 ppm**	
	_	_	_	_	_	_	_	_
Parent data								
mortality			all F0 and F1 survived					
clinical findings	hair loss, scabbing and red material on skin, soft stool,tan feces, decreased defecation, red and swollen ears, ocular opacity, exophthalmia,lacrimation.							
Reproductive performances:								
Fertility indices (%): Fo	86.7	90.0	86.7	90.0	90.0	93.3	93.3	93.3
F1	76.7	76.7	80	83.3	86.7	86.7	93.3	93.3
Mating indices (%) Fo	90.0	96.7	93.3	96.7	96.7	96.7	93.3	93.3
F1	90.0	93.3	90.0	93.3	93.3	93.3	93.3	96.7
N°._ not siring a litter Fo	4		4		3		2	
F1	7		6		4		2	
N°.non gravid _ Fo/F1		3/7		3/5		2/4		2/2
N°. no mating_ Fo/F1		1/2		1/2		1/2		2/1
food consumption F0						îwk 0è1	î wk 0è 4; 8è 10; 14, 18è 20	î wk 0è11
F1							î wk 18è 39	î wk 18è 31, 34, 37è 39
F1 during gestation								î day 0-20
F1 during lactation						î day 1-21		î day 0-21
body weight F0							î wk1,2,3	î wk 1è 10 î wk 17è 20
F1							î wk 18è39	î wk 18è32, 37è39
F2					îwk 39		îwk 38,44	îwk 38,44
body weight gain F0			îwk 2, 6		îwk1, 2,3, 6	îwk2, 8	îwk ,0,4, 6	îwk 0,6

dose/endpoint	0 ppm		72 ppm		207 ppm		540 ppm	
	_	_	_	_	_	_	_	_
F1							îwk 18, 20è22, 33,35	îwk 18, 23è25
body weight during gestation F0								îday 0,7, 10, 14, 20
body weight changes during gestation F0								îday 10-14
F1								îday 0-20
body weight during lactation F0								îday 1,4,7,14
body weight changes during lactation F0								îday 14-21
F1								îday 1-21
organ weight								
F0								
brain								î a ; ì r 5%
liver							ìr, 9%	
F1								
brain							î a; ìr	ìr
kidneys						î a 5%	î a	î a
epididymus and testes							ìr	
ovaries						ìr 8%		
F2								
brain							ìr	
pup indices								
sex ratio F1	199M/168F						152M/184F	
viability lactation day 1			î 5.8 % F1					
mean pup weight F1							î day 14	
mean pup weight F2							î day 1, 4, 14, 21	

î (decrease) or ì(increase) significantly different from control group using Dunnett's test for parent data, live litter size; pup survival,sex ratio ; number dead using Chi-square. a: absolute; r: relative

All the F0 parental animals survived to the scheduled necropsy. Clinical signs were not related to ziram intake.
Parental toxicity in the F0 and F1 generations was exhibited at a concentration of 540 ppm by inhibition of body weight gain and reduced food consumption.
No parental toxicity was observed at concentrations of 72 and 207 ppm.
Reproductive parameters (fertility, mating, days between pairing and coitus, gestation and parturition) were not adversely affected by ziram administration .
At the scheduled necropsies, no macroscopic internal findings nor microscopic lesions related to ziram administration were observed in any F0 and F1 tissues. Necropsy findings for the F1 and F2 pups that died or were euthanized were not suggestive of any correlation with parental treatment.
Neonatal toxicity was expressed at a concentration of 540 ppm by slightly reduced pup body weights (F1 and F2).
No neonatal toxicity was observed at 72 and 207 ppm.

An additional objective of this study was to investigate the potential of ziram to cause functional and morphological changes to the nervous sytem of the developing rat (F2 litters) following treatment of the F0 and F1 generations.
Various indicators of physical and functional development, as well as behavioral responses, of the selected F2 treated pups in the developmental neurotoxicity phase were comparable to the control group values. At the postnatal day 11 and 70 of the neuropathological examinations, no gross or microscopic ziram-related lesions were observed in the selected F2 pups.

Conclusion:

NOAEL maternal systemic toxicity = 207 ppm = 10 mg/kg bw/d.
NOAEL neonatal toxicity = 207 ppm not taking into account the small organ weight modifications = 10 mg/kg bw/d.
NOAEL reprotox = 540 ppm = 25 mg/kg bw/d
NOAEL neurotoxicity = 540 ppm

Guidelines:
Experimental protocol in compliance with test method B.5 of Directive 87/302/EEC.
GLP status : yes (no attest of competent authority)
Material and methods :
30 sprague-Dawley Crl:Cd Br /sex/dose received in the diet ziram(technical, lot V528/8331AA; 97.8%) at 60, 180 (fortified to 72 and 207 ppm respectively due to the instability in the diet) and 540 ppm. Parental animals received the diet for at least 70 days prior to pairing and throughout all subsequent phases of the study until termination of the generation.

Compound consumption (mg/kg/day)

theoretical dietary level (ppm)	males		females			
	prior breeding	after breeding	prior breeding	gestation	lactation	after weaning
72	5	3	6	5	12	5
207	15	10	17	13	33	15
540	37	25	43	33	85	37

B.5.6.2.1 Teratogenicity test by the oral route in the rat (Annex IIA 5.6.2)

- Rat, oral administration at 1, 4, 16 and 64 mg/kg bw/day, day 6 to 15 of pregnancy (Smith et al., 1990, task force study)

Findings :
Range finding study:
80 mg/kg bw /d was too high for the main embryotoxicicity study : symptoms were post-dosing salivation, hair loss, decreased food consumption, slight body weight loss to day 8 of pregnancy , increased post implantation loss and reduced litter and mean foetal weight.
- 20 mg/kg bw/day: hair loss in occasional animals, decrease in food consumption, reduced weight gain to day 8 of pregnancy, slightly reduced litter and mean foetal weight.
- 5 mg/kg bw/d: marginal reduction in food consumption, slightly reduced weight gain to day 8 of pregnancy without adverse effect on mean litter parameters. Treatment at all dosages did not produce any obvious structural abnormalities among foetuses at examination on day 20. There was however an increased incidence of small foetuses (<2.5g) 3/98, 0/113, 6/95 and 31/116 in group 1 to 4 respectively. At 80 mg/kg bw/d, 8/10 litters were affected and this was reflected in the significantly reduced mean foetal weight.

Main study:
Main findings are described in table B.5.6.2.1-1.
The overall incidence of malformed foetuses at 64 mg/kg bw /d was identical to the control.
The incidence of foetuses with *visceral anomalies* was marginally higher at 16 and 64 mg/kg bw/day compared with controls, this increase was not dosage-related but 5/20 and 5/16 at 16 and 64 mg/kg bw/d respectively showed thinning of the diaphragm. Although a high proportion of foetuses at the two top doses showed the same visceral anomaly this is thought unlikely to be treatment-related as no foetuses at these dosages showed diaphragmatic hernia.
Skeletal variants: the marginally higher incidence of foetuses at 64 mg/kg bw/d with unossified sternebrae was probably related to the lower foetal weight

Table B.5.6.2.1-1 : Teratogenicity study by the oral route in the rabbit

Endpoint/dose (mg/kg bw/d)	**0**	**1**	**4**	**16**	**64**
Dams					
mortality:		no mortalities			

Endpoint/dose (mg/kg bw/d)	**0**	**1**	**4**	**16**	**64**
clinical signs:					
salivation				2/25	8/25
hair loss					6/25
body weight gain					retarded to d 16
body weight loss				to d 8 in 20/24	to d 8 in 13/23
mean food intake				î	î
mean water consumption				ì	ì
pregnancy rate(%)	92	96	88	92	96
pre-implantation loss		no treatment related effect			
Foetus and litter : developmental effects					
mean foetal weight					î
mean litter weight					î
incidence malformed foetuses	1.6%	0.7%	0.4%	0.4%	1.4%
incidence of foetuses with vsiceral anomalies	8.3%	4.9%	6.9%	14.9%	12.2%
thinning of diaphragm (n° fetus affected)			1/9	5/20	5/16
skeletal anomalies		no effect			
skeletal variations: unossified sternebrae %	59.8	59.1	51.3	54.6	68.2 marginal increase

Statistically significant increase (ì) or decrease (î) (Williams test; Kruskal-Wallis 'H' statistics)

Conclusion : No teratogenic effect was seen.
NOAEL maternal toxicity : 4 mg/kg bw/d
NOAEL fetal toxicity = 16 mg/kg bw/d
NOAEL developmental toxicity : 4 mg/kg bw/d taking into account the marginal increase in visceral anomalies observed at 16 and 64 mg/kg bw/d.

Guidelines:
Experimental protocol not fully in compliance with method B. of Directive 87/302/EEC or OECD guideline 414 (1981).
GLP status: attest of study director and QAU (no attest of competent authority)
Deviation from the official protocol: pregnant females arrived at the laboratory 4 days before beginning of exposure.
Material and methods :
- Range finding study:
10 pregnant rats (Crl:CD(SD) BR VAF/Plus strain)/dose received by oral gavage ziram (technical, b.n°.8331 AA; 98.9%) at 5, 20 or 80 mg/kg bw on day 6 of pregnancy up to and including day 15 of pregnancy. A control group received the solvent only (1% methyl cellulose).
- Main study:
25 pregnant rats (Crl:CD(SD) BR VAF/Plus strain)/dose received by oral gavage ziram (technical, b.n°.8331 AA; 98.9%) at 1, 4, 16 or 64 mg/kg bw on day 6 of pregnancy up to and including day 15 of pregnancy. A control group received the solvent only (1% methyl cellulose).

B.5.6.2.2 Teratogenicity test by the oral route in the rabbit (Annex IIA 5.6.2)

- Pregnant rabbit, 3, 7.5, 15 mg/kg bw/d (Barker, 1985 and 1986, task force study)

Findings :

Range-finding study:
At 20 mg/kg bw/d, 2 animals died and one aborted. Marked reductions in weight gain and food intake, particularly at the start of the treatment. Increased post-implantation loss, reduced litter size and litter weight and foetal weight were different from expected values.
At 10 mg/kg bw/d: slight temporary reductions in weight gain and food intake .
At 5 mg/kg bw/d: one incidental death was observed and one was observed in the control group.

Conclusion : Based on the maternal toxicity observed at 20 mg/kg bw/d, the high dose level for the main study should be less than 20 mg/kg bw/d.

Main study:

Table B.5.6.2.2-1 :Teratogenicity study by the oral route in the rabbit

Endpoint/dose (mg/kg bw/d)	**0**	**3 mg/kg bw/d**	**7.5 mg/kg bw/d**	**15 mg/kg bw/d**
Dams:				
mortality	1		2	1
clinical signs	no indication of an effect			
body weight			î day 10-13	î day 9-13**
body weight gain			î day 8-16	î entire gestation period
food intake				î day 7-19**
mean gravid uterus weight (kg) no stats.	0.46	0.44	0.39	0.37
% pre-implantation loss	8.5	8.2	19ì	6.5
early intrauterine deaths (number)	12	3**	4	5
post-implantation loss (%)*	8.6	4.1	7.2	16.9
Foetus and litter :				
mean litter weight				î **
mean foetal weight				î not sign.
litter size			î	î
mean crown/rump length (mm)	98.7	98.5	99.2	96.5
External and visceral defects: number fetuses affected (%)				
major defects	0 (0)	2 (1.4)	0 (0)	0 (0)
minor defects	11(8.6)	4 (2.9)	6 (5.8)	8 (7.5)
variants	25(19.5)	17(12.1)	14 (13.6)	13(12.1)
Skeletal defects : number fetuses affected (%)				
major defects	2 (1.6)	6 (4.3)	0 (0.0)	4 (3.7)
minor defects***	76 (59.4)	82 (58.6)	60 (58.3)	57 (53.3)
variants	123 (96.1)	114 (81.4)	97(94.2)	97 (90.7)

* Post implantation loss was higher in top dose than controls but equals the highest value seen in control groups during the six most recent studies.The increase is partially due to a high incidence of late intrauterine deaths in 1 animal.
** statistically significantly different from control (analysis of variance, 't' test ; Kruskal-Wallis, Wilcoxon's tests) increase (ì) or decrease (î)
*** historical control data = 39.5%

Mortality was considered incidental to treatment.

Treatment with ziram at 15 mg/kg bw/d during organogenesis elicited maternal toxicity characterized by significant reduction of body weight gain and food intake. Foetal weight was also adversely affected and an association with increased post-implantation loss could not be dismissed. There was no indication, however, of a teratogenic effect at this dose level.
Slight retardations of body weight gain and food intake were also observed at 7.5 mg/kg bw but there was no adverse effect on embryonic or foetal development. The reduced litter size was considered as a result of the high pre-implantation loss. This effect is considered as unlikely to have been associated with treatment as implantation would be expected to occur before the start of dosing on day 7 of gestation.

There was no maternal or foetal effect of treatment, at 3 mg/kg bw/d.

Major defects observed in foetuses (number affected) were:
- In control group: multiple vertebral and rib defects (2)
- In group 3 mg/kg bw/d : multiple vertebral and rib defects (2), multiple head defects including microstomia, cleft palate, brachygnata, cyclopia, hydrocephalus (1), microphthalmia and sternebral defects (1) and multiple vertebral defects (2)
- In group 7.5mg/kg bw/d : no major defects were observed.
- In group 15 mg/kg bw/d: multiple vertebral and rib defects (2), and interparietal absent (2).

The observed incidence of major skeletal anomalies was higher than the expected values and may have been adversely influenced by 2 males which sired 5 of the 87 foetuses showing major vertebral/rib defects. No effect of treatment is concluded, however, as these defects are known to occur spontaneously in this strain of rabbit and their intergroup distribution was not related to dose.

Conclusion:
There was no teratogenic effect.
NOAEL maternal toxicity = 3 mg/kg bw/d
NOAEL developmental toxicity = 7.5 mg/kg bw/d

Guidelines :
Experimental protocol in compliance to the method B of the Directive 87/302/EEC or OECD guidelines 414 (1981).
GLP status : authenticity attest and QAU attest (no attest of competent authority).
Material and methods :
Range-finding study:
5 mated female New Zealand white rabbits/dose were given ziram (technical, 98%, b.N°:Z-1461) orally, from day 7 to 19 of gestation at dose levels of 5, 10 or 20 mg/kg bw/day. Control animals received the vehicle (1% aqueous methyl cellulose)
Main study:
16 mated female New Zealand white rabbits /dose were given ziram (technical, 98%, lot P15/62,06-85) orally, from day 7 to 19 of gestation at dose levels of 3, 7.5 or 15 mg/kg bw/day. Control animals received the vehicle (1% aqueous methyl cellulose)

- Additional information from open literature:

- Rats, 12.5, 25, 50 or 100 mg/kg bw/d, gastric intubation, during the first 5 days of pregnancy or during organogenetic period (Giavini et al., 1983)

Findings:
Preimplantation study : none of the females showed clear signs of toxicity, but at 50 mg/kg bw/d, body weight gain was significantly reduced during treatment.
A reduction in the live fetuses mean weight in group 50 and 100 mg/kg bw/d was reported. At 100 mg/kg bw/d, a sharp decline in the average number of sternal ossification centers was observed and higher rate of minor visceral anomalies in this group. A substantial increase in major malformations occurrence in the group treated with the lowest dose.This effect is regarded as an accidental episode that cannot be ascribed to treatment. The weight reduction in fetuses at term has been shown to be accompanied by a delayed ossification. This result cannot be ascribed to patent toxic effects on mothers, since mother weight gain during postimplantation is not significantly different from the control values. This retardation in growth at term could be ascribed to a retardation in implantation caused by ziram.
Treatment does not alter the average number of implantations, number of live fetuses, pre- and postimplantation losses.

Table B.5.6.2.2-2 : Preimplantation study by the oral route in the rat

	Dose (mg/kg bw/d)			
	0	**25**	**50**	**100**
Mated females	15	15	15	15
Pregnant females	14	15	15	14
Maternal bw gain :				
days 1-5	12.6 ± 7.5	5.9 ± 10.9	1.4* ± 10	6.8 ± 16
Days 6-21	97.4 ± 15.8	99± 19.4	84 ± 23.7	99.3 ± 22.2
N° corpora lutea	215	215	239	215
N° implantations	189	208	202	201
mean	13.5	13.8	13.4	14.3
Preimplantation loss %	12.1	3.2	15.5	6.5
N°.live fetuses	175	196	184	184
mean	12.5	13	12.2	13.1
Postimplantation loss %	7.4	5.7	8.9	8.4
mean fetal weight	3.94	3.88	3.64*	3.65*
malformed litters	1	3	2	1
Malformed/examined fetuses	1/175	7/196	2/184	2/184
Average N°. ossified sternebrae	5.81 ± 0.33	5.61 ± 0.67	5.62 ± 0.51	5.33 *± 0.66

Teratogenic study: A clear toxic effect was observed in all treated groups. Ziram has a low teratogenic properties that becomes effective at dosages that are lethal to 50% of mothers (100 mg/kg bw/d). Lower doses have not brought about clear teratogenic effects although they are known to be toxic for pregnant females. The dose-related reduction in the mean weight of live fetuses as well as the lower degree of ossification, shown by the average number of sternal ossification centers at the higher dose levels, is most probably due not so much to a direct effect of the substance on the product of conception as to mother suffering.

Table B.5.6.2.2-2 : Teratogenicity study by the oral route in the rat.

Endpoints	**Dose (mg/kg bw/d)**				
	0	**12.5**	**25**	**50**	**100**
mated **females**	13	13	13	13	11
pregnant females	11	9	10	11	5
dead females	0	1	0	2	6**
maternal bw gain day 6-21 (g)	104.8±9.4	88.3*±11.3	88.1*±27.5	68.5*±24.3	34.4*±35.5
mean implantations	13.8	13.4	13.0	12.5	14
postimplantation loss %	1.3	4.9	3	6.5*	9.6*
N°. live **fetuses**	150	115	126	129	66
mean live fetuses	13.6	12.7	12.6	11.7	13.2
Mean fetal weight	3.95	3.82	3.58*	3.38*	2.48*
malformed litters	0	0	0	1	2
malformed/examined fetuses	0/150	0/115	0/126	1/129	5/66*
Average N° of ossified sternebrae	5.63 ± 0.41	5.66 ± 0.48	4.95 ± 1.74	5.07 ± 0.46*	3.48 ± 1.61*

*** P<0.05**

Conclusion: At 100 mg/kg bw/d, a slight dismorphogenic effect was reported in the teratogenicity study.
Maternal toxicity was reported at all doses and embryotoxic effects appeared at 25 mg/kg bw/d.
NOAEL maternal toxicity < 25 mg/kg bw/d
NOAEL embryotoxicity = 12.5 mg/kg bw/d
NOAEL teratogenicity = 50 mg/kg bw/d

Comment : In this study, the authors consider that in the preimplantation study, maternal body weight gain is only significantly affected at 50 mg/kg bw/d. However, from the table B.5.6.2.2-2, it appears that maternal body weight gain is probably also affected at the lower dose levels.

Material and methods:
Female CD rats were matted . The day the vaginal smear proved positive was considered the first day of gestation. Technical grade Ziram (gift of Montedison) was given orally as an oral supension in 2% arabic gum by gastric intubation.
Preimplantation study : 60 females put in 4 groups treated with 0, 25, 50 and 100 mg/kg bw/d from day 1 to 5 of gestation.
Teratogenic studies: 36 females put in 5 groups received 0, 12.5, 25 , 50 and 100 mg/kg bw/d from day 6 to 15 of gestation.
(study protocol comparable to recommended protocol)

B.5.6.2.3. Summary of reproductive and development toxicity :

Ziram did not affect reproductive performance in the 2-generation rat study at the doses studied. Parental toxicity was observed in the F0 and F1 generations at the dose of 25 mg/kg bw (food concentration 540 ppm), e.g., inhibition of body weight gain and reduced food intake. At the same dose neonatal toxicity was expressed, e.g., slightly reduced pup body weights (F1 and F2).

Teratogenic effects, with regard to embryofoetal development and morphology, were not observed at doses that did not also produced maternal toxicity in rats. In rabbits, maternal toxicity was observed at 7.5 mg/kg bw/d but no teratogenic effects were observed.

From open literature, ziram has a low teratogenic effect that only becomes effective at dosages that are lethal to 50% of the mothers (100 mg/kg bw/d).

Table B.5.6.2.3-1: Summary of reproductive and development toxicity

Study	**NOAEL** mg/kg bw/d	**LOAEL** mg/kg bw/d	**Critical effects**	**Purity (%) batch number**	**References**
2 generations, oral, rat	systemic maternal tox.: 10 neonatal tox : 10 reproduction : 25	25 -	alteration of weight parameters. -	technical, lot V528/8331AA 97.8%	Nemec, 1996
terato, oral, rat	-maternal toxicity: 4 - developmental: 4	16 16	salivation, î food consumption,bw loss, retarded bw gain î mean foetal mean litter weight marginal ì visceral anomalies	technical, b.n°.8331 AA; 98.9%)	Smith et al., 1990 task force study
terato, oral, rabbit	-maternal toxicity :3 -developmental :7.5	7.5 15	î bw gain, food intake and mean gravid uterus î litter and foetal weight, î crown-rump length	technical, 98%, b.N°:Z-1461	Barker, 1986 task force study

B.5.7 Neurotoxicity (Annex IIA 5.7)

As signs of autonomic nervous system effects were observed after acute oral administration of high doses of ziram, additional studies were realized.

B.5.7.1 Neurotoxicity following acute exposure in rats. (Annex IIA 5.7)

- Rats, oral administration of a single dose at 15, 300 and 600 mg/kg bw (Lamb, 1994 , task force study)

Findings:
Functional Observation Battery (FOB) and motor activity evaluations were performed during pretest, at the time of peak (approximatively 4 hours post-dosing or day 0) and on day 7 and 14.
In general, the occurrence of clinical signs was limited to the first week of study. At 15 mg/kg, the only possible treatment related clinical sign was rales observed on day 1. At 300 and 600 mg/kg bw, absolute brain weight was reduced. Similar effects as those reported after acute oral toxicity study (point B.5.2) were reported.
No treatment related microscopic lesions were observed in any of the central or peripheral nervous sytem tissues examined from the 5 animals/sex in the 600 mg/kg group.

After FOB tests, response occurred approximatively 4 hours after exposure and was transient in nature. Remarkable signs observed during the study day 14 FOB evaluations were generally limited to 2 males in the 300 mg/kg bw group. Thoses signs observed for one or both included: altered posture and palpebral closure during the home cage observations, altered palpebral closure changes in the respiratory character, changes in mucous membrane, skin and eye colour, change in fur appearance, crusty deposits around nose and mouth, changes in eye prominence during handling observations; markedly decreased body temperatures and weights during the physiological observations. These findings were not observed during the study day 7 FOB evaluation with the exception of respiratory rales for both males. Moreover, some of these signs were not observed at day 0 FOB for these animals or the 600 mg/kg bw group males and females at any evaluation period.
The only possible effects on FOB parameters in the 15 mg/kg bw group, observed during day 0 included altered posture and slight impairment of gait.
Sensory and neuromuscular observations were performed during open field tests. Impairment of walk and tiptoe gait, impaired mobility, were observed at 300 and 600 mg/kg bw and altered tail pinch and touch was noted at 600 mg/kg bw. Body temperature was reduced on day 0, 7, and 14 from 300 mg/kg/bw up to 600 mg/kg bw. Total activity was reduced.

Conclusion:
The neuromuscular and CNS activity domains appeared to have been slightly affected at a dose level of 15 mg/kg.
NOAEL neurotoxicity = 15 mg/kg bw
NOAEL systemic toxicity = 15 mg/kg bw

Guidelines:
This study protocol is not fully in compliance with OECD guideline 418 (1984)
Deviation from the official protocol: rat instead of hens ; 14 days observation and not 21. Tests were performed only once a week and not twice. Histopathology was performed on solely 5 rats/sex from control group and maximal dose. No positive controls.
GLP status :yes
Material and methods:
12 rats (Sprague-Dawley, Crl:CD BR) received ziram (lot number V528/8331AA; 97.8%)) by gavage at 15, 300 or 600 mg/kg bw . A control group received the vehicle, corn oil.

B.5.7.2 Neurotoxicity following chronic exposure in rats. (Annex IIA 5.7)

- Rat, oral administration in diet, 72, 207, 540 ppm during 90 days (Lamb, 1993, task force study)

Findings:
One male in the 72 ppm group was found dead on day 14. This animal displayed hypoactivity, laboured respiration. A large mass was found in the thymus gland. This death was not considered to be related to treatment.
There were no apparent clinical signs observed for the surviving animals.
No ziram related effects indicative of neurotoxicity were apparent when F O B and Locomotor Activity evaluations were performed.
Brain neuropathy target esterase (NTE) was significantly inhibited at the top dose. No remarkable differences from the control group were observed in mean plasma, red blood cell and brain cholinesterase values at any dose of ziram during study weeks 3, 7, and 13. Absolute and relative brain weights were normal.
No treatment related neuropathological lesions were observed at the microscopic examination of perfused tissues for 5 animals/sex in the 540 ppm group.

Conclusion :
Repeated dosing of ziram in the rat, at doses producing systemic toxicity, did not induce behavioural and neuronal toxicity. The toxicological significance of the moderate inhibition of the brain NTE is not clear. Neurotoxicity, more especially OPIDN (organophosphate-induced delayed neuropathy) has been linked to profound NTE inhibition, accompanied by ageing of the agent-enzyme complex.

NOAEL neurotoxicity = 540 ppm = 34 mg/kg bw/d
NOAEL systemic toxicity = 207 ppm = 14 mg/kg bw/d

Guidelines:
This study protocol was partly in compliance to the OECD guideline 419 (1984).
Deviation from the official protocol :Rats were used instead of hens. Tests were performed on weeks 1, 3, 7, 12 and not once a week. Histopathology was performed only on half of the animals .
Material and methods:
10 Sprague-Dawley(Crl: CD BR) rats/sex/dose received ziram (lot n°.V528/8331AA; 97.87%) in the diet at 0, 72, 207 or 540 ppm during 90 days. In each group, 5 animals/sex/dose were allocated to cholinesterase/neurotoxic esterase (NTE) evaluations and 5 animals/sex/dose to a neuropathology evaluation.
Converted dose for males : 5, 14, 34 mg/kg bw/d
Converted dose for females : 6, 16, 40 mg/kg bw/d
GLP status: yes

B.5.7.3 Summary of neurotoxicity (Annex IIA 5.7).

Based on the results of the FOB and motor activity evaluations, one or more parameters in each of the following functional domains were affected by a single oral administration of ziram at 300 and 600 mg/kg: autonomic, neuromuscular, sensorimotor, CNS activity and physiological. The neuromuscular and CNS activity domains appeared to have been slightly affected at a dose level of 15 mg/kg.
It has been suggested that these neurotoxic effects might be due to the metabolite CS2 (Harry et al., 1998).
In the subchronic neurotoxicity study , no ziram related effects indicative of neurotoxicity were apparent.

Table B.5.7.3-1: Neurotoxicity of ziram

Type of study	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Critical effects	Batch n°, purity	References
acute neurotoxicity	15	300	several clinical signs, body weight alterations	lot number V528/8331AA; 97.8%	Lamb, 1994 task force study
90 days, oral, rat neurotox.	systemic tox. :14 neurotox.: 34	34 -	î mean brain neurotoxic esterase -	V528/8331AA; 97.87%	Lamb, 1993 task force study

B.5.8 Further toxicological studies (Annex IIA 5.8)

B.5.8.1 Toxicity studies on metabolites/contaminants (Annex IIA 5.8.1)

Tetramethylthiourea (TMTU) is an impurity present at very low levels (0.1g/kg) in ziram. TMTU, was added to the diet of male and female rats for up to 2 years at levels of 0, 30 and 300 ppm. At the top dose, rats had a lower body weight and consumed less diet. A slight anemia was found. Serum thyroxine was lower. Thyroids were enlarged (after 3 months) and there was an increase in follicule lumen size, loss of colloid staining, hypertrophy and hyperplasia of follicular cells together with hyperhemia. 28% of 29 females high dose, 18 to 24 months on the test, developed follicular cell carcinomas with no such tumors in 26 comparable controls (Stula et al., 1979).

B.5.8.2 Additional studies (Annex IIA 5.8.2)

The effects on the thyroid became critical in the assessment of ziram toxicity and further explanations will be needed to clear if ziram treatment may increase or decrease the proliferating lesions of C-cell in the thyroid.

B.5.9 Medical data (Annex IIA 5.9)

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

Ziram is normally not produced as only product over some time. So all personnel is exposed to several products at a time. All products concerned are of the dithiocarbamate group (fungicides : ferbam, metham sodium, thiram,...).
Blood samples (RBC, Hb, WBC, sedimentation rate, GOT, GPT, GGT, bilirubine, alacaline phosphatase, Fe and Cu) are taken at yearly intervals and tests for lung function are performed every six months(Vital capacity and Forced expiratory volume in one second). WBC-analyses reveal regularly a limited rise in neutrophil-values which disappear during non-exposure. None of the tests show other systemic deviations.
Skin irritation is seen rather sporadically in the UCB plant due to sufficient hygienic conditions and the use of personal protection clothes (Dr Mahy, UCB).
No other symptom has been recorded.

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

Workers which were exposed to 1.6-2.2 mg/m3 of ziram had increased incidence of nonspecific diseases of the upper respiratory tract such as rhinitis, laryngitis and pharyngitis, bronchitis, and emphysema of the lungs.
Ziram is a strong contact allergen in humans which cross-reacts with a variety of other thiurams and dimethyldithiocarbamates (Savolainen, 1990)

B.5.9.3 Observations on exposure of the general population and epidemiological studies. (Annex IIA 5.9.3)

No epidemiological studies were performed by UCB-sa. No major accidents which involved population were reported up to now.

B.5.9.4 Clinical signs and symptoms of poisoning and details of clinical tests (Annex IIA 5.9.4)

No data available in the dossier.

Additional information from open literature:

Occupational exposure to dithiocarbamate formulations mainly leads to direct skin contact and dermal absorption, inhalatory exposure may also play a significant role. Dithiocarbamates are, in general, readily but incompletely absorbed from the gastrointestinal tract. (Liesivuori and Savolainen, 1994)

Acute poisoning:
Acute poisoning is unlikely unless very large quantities of the material is ingested. Toxicity may be expressed by convulsions, transient kidney failure and/or affect thyroid function. In man, accidental ingestion has elicited nausea, emesis, diarrhea, headache, ataxia, confusion and fatigue in mans. Convulsions have been described after acute occupational exposure (Liesivuori and Savolainen, 1994)

Chronic poisoning:
- The main chronic toxicity of dithiocarbamates described in man concerns a goitrogenic effects and quite strong sensitizing properties. Subjects with disorders of thyroid or kidney function should be carefully evaluated before employement at workplaces where significant exposure to dithiocarbamates is possible (Liesivuori and Savolainen, 1994).
- Following chronic inhalation exposure, similar effects are noted as observed following acute exposure; besides that, neuronal and visual disturbances were also reported following long-term exposure. In cultured lymphocytes of workers exposed to Ziram , higher frequency of abnormal chromosomes or chromatids was reported.
- Following heavy exposure of the skin, dithiocarbamates may elicit dermatitis. Repeated contact may also induce sensitization reactions in some individuals. Workers with severe skin diseases, especially eczema or allergic dermatitis, should avoid contact with and exposure to dithiocarbamates.
- Following eye contact, repeated and prolonged exposure to Ziram may cause conjunctivis .
- Following ingestion, signs and symptoms in experimental animals are nonspecific : fatigue, hair loss, reduced weight gain, and shortened life span. All of theses effects were dose-related. At higher dose levels following long-term exposure, neurological signs such as paralysis of the hind legs are common findings. Repeated exposure may lead to hyperplasia of the thyroid (Pestline, 1990).
- Dithiocarbamates may also affect the central nervous system via their metabolites. Examples include CS_2, and H_2S, all of which are well-known neurotoxins. H_2S is also a potent inhibitor of oxidative phosphorylation which causes histotoxic hypoxia in the brain, heart, and kidney cells. The metabolic reduction of the disulfide bond may cause the inhibition of several hepatic enzymes. This may explain the capacity of dithiocarbamates to inhibit their own metabolism as well as the metabolism of several other compounds such as ethanol.
- Thiram and other dithiocarbamates have an inhibitory effect on ethanol hepatic metabolism and following alcohol ingestion, exposed individuals may experience adverse reactions, such as flushing (Pestline, 1990; Liesivuori and Savolainen, 1994)
- under mildly acidic conditions, such as those existing in the human stomach, ziram may react with nitrite to form N-nitrosodimethylamine, which has been shown to be carcinogenic in several animal species.

Biological monitoring of exposure to dithiocarbamates:
There are presently no widely accepted methods for biological monitoring of exposure to dithiocarbamates, mainly due to the lack of knowledge of their pharmacokinetics in humans, and the lack of adequate analytical chemistry methods.
To assess high-level exposures, measurement of urinary carbon disulfide (CS_2), which is a metabolite of almost all dithiocarbamates, has been suggested. Thiurams are partly excreted unchanged, they can be assayed in urine samples by gas and high-pressure liquid chromatography. Dimethylcarbamate is one of the metabolic end products for which several analytical methods have been developed. They have, however, not proved useful. Urine samples from exposed workers should be taken in the morning following the cessation of the xposure. Samples should be kept cold,and sent to the laboratory without delay. There are no generally accepted biological exposure indices established for dithiocarbamates (Liesivuori and Savolainen, 1994).

B.5.9.5 First aid measures - Therapeutic regimes (Annex IIA 5.9.5)

Skin contact: if the compound has been spilled on the skin, it should be immediatly removed so that dermal exposure can be effectively prevented. All contamined clothing should be immediatly removed, and the skin washed with soap and water without any delay. Liesivuori and Savolainen, 1994).

Eye contact: if the fungicide has contamined the eyes, they should be washed with clean water at least for 20-30 min. (Liesivuori and Savolainen, 1994). Occasionally lifting the upper and lower lids until no evidence of the chemical remains is recommended. Seek medical attention.

Ingestion: in case of ingestion of significant quantities of the fungicide, and if emesis did not already occurred and if the victim is fully alert, and provided that local medical doctrine includes emesis as an emergency treatment, syrup of ipeca may be administered, followed by 1-2 glasses of water (older than 12 years : 30 ml; children under 12 years of age : 15 ml). If consciousness declines or vomiting does not occur within 15 min, empty the stomach by intubation, aspiration, and lavage, using all available means to avoid aspiration of vomitus. Following aspiration of the stomach and washing with isotonic saline or sodium bicarbonate, 30-50 g of activated charcoal in approximately 100 ml of water needs to be instillated through the stomach tube to limit absorption of the remaining toxicant. If the irritation properties of the toxicant fail to produce a bowel movement in 4 hours, sodium or magnesium sulphate must be administered as a cathartic : 0.25 g/kg bw in 30-200 ml of water. Glucose-containing fluids should be administered intravenously to accelerate excretion of the toxicant. Medical attention should be sought immediatly.

Inhalation: after inhalation exposure, the subject should be removed out of the contamined environment. If breathing has stopped, artificial respiration should be performed. The person should be kept warm and at rest. Treatment should be symptomatic and supportive, and medical attention should be sought (Pestline, 1990; Liesivuori and Savolainen, 1994).

Advice on treatment: No specific antidotes are available against dithiocarbamate poisoning. In severe poisoning, anticonvulsivant drugs such as diazepam can be given intravenously. Hydration of the victim with physiological solution, and cortisone treatment may be useful. In most cases, symptomatic treatment is adequate. Cortisone-containing emulsions may be needed in the treatment of dithiocarbamate-induced skin sensitization. (Liesivuori and Savolainen, 1994).

B.5.9.6.1. Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion (Annex IIA 5.9.6)

no data available.

B.5.9.6.2 Expected effects and duration of poisoning as a function of varying time periods - between exposure or ingestion and commencement of treatment (Annex IIA 5.9.6)

no data available.

B.5.10 Summary of mammalian toxicology and proposed ADI, AOEL and drinking water limit (Annex IIA 5.10)

Metabolism:
In rats, Ziram is well absorbed from the gastro-intestinal tract, within 48 h after oral administration for 58-61% after a single low dose, for 60-69% after a single high dose and for 71-75% after a repeated low dose. Which means an overall oral absorption of 66%.

Partial inactivation, by a first-pass hepatic elimination, is suggested by the fact that, after inhalation, signs of systemic neurotoxicity were observed after 30 times lower doses than after oral ingestion.

After repeated exposure, recovery of radioactivity in tissues and carcass was low 7 days after exposure, suggesting that the compound does not accumulate.
In the dossier, metabolites were not identified and no metabolic scheme was proposed by the notifier. This point has to be addressed by the notifier. According to the JMPR (1996), Ziram is metabolized to dimethyldithiocarbamate and excreted as S-glucuronide conjugate. Other metabolic products include carbon disulfide, sulfate and dimethylamine .

The most important elimination route of the radioactive marker was via expired air (36 to 53% of the dose administered), the majority of which was expired during the first 48 h post-dosing. Urinary excretion represented 17% (high dose within 24 h) to 35% (repeated low dose within 24 - 48 h). Faecal excretion represented 9 to 18.5% and decreased with increasing the dose.

Acute toxicity:
Ziram is harmful when swallowed, and very toxic by inhalation. It is not a skin irritant but produces severe ocular lesions, skin sensitization may occur.

Based on the results of the FOB and motor activity evaluations, one or more parameters in each of the following functional domains were affected by a single oral administration of ziram at 300 and 600 mg/kg: autonomic, neuromuscular, sensorimotor, CNS activity and physiological. The neuromuscular and CNS activity domains appeared to have been slightly affected at a dose level of 15 mg/kg. It has been suggetsted that these neurotoxic effects might be due to a metabolite CS2 (Harry et al., 1998).

Target organs, as indicated by macroscopic changes , are liver and lungs.

Genotoxicity:
Ziram, after metabolic activation, induced base-pair substitution in strain TA100 of *S. typhimurium*. A gene mutation test in mammalian cells was not supplied. This point has to be addressed by the notifiers . Ziram did not induce chromosome aberrations in CHO cells *in vitro*, and in mouse bone marrow cells and spermatogonia *in vivo*. It did not induce micronuclei in peripheral erythrocytes after sub-chronic exposure *in vivo*, although it is not clear whether toxic doses were reached. In another study, ziram did not induce micronuclei in mice bone marrow after oral exposure. Ziram did not induce DNA repair activity in hepatocytes in culture.
In the open literature, positive results have been published based on different methods. The reliability of these results is, however, questionable. Therefore, our conclusions are based on test results obtained according to officially accepted test methods.
Ziram is not genotoxic.

Short-term, repeated dose toxicity:
Ziram decreased in rats and mice, body weight gain, food intake and food efficiency. Dogs presented a subdued behaviour and/or unsteady gait, with slight alterations in body weight and food efficiency, increase in liver weight, they vomited and had diarrhoea.

In the rat, T4 serum level showed a dose related reduction, T3 and TSH were not significantly affected. Moreover, minor, but consistent, changes in biochemical parameters were observed (îtotal protein , albumin, Ca^{++}, RBC and ì urea nitrogen , creatinine and MCHC).

In the rat, the target organs were liver and kidneys, as shown by the presence of degenerative alterations. In mice, toxic effects were more diffuse and no specific target organ could be determined. Dogs showed an increased liver weight.

In the subchronic experiment (90 day study), the non-glandular stomach was the target organ, with minimal epithelial hyperplasia in rats, and epithelial hyperkeratosis and minimal epithelial hyperplasia of the limiting ridge in the stomach of mice.

In dogs, the target organs were liver and spleen, with at the start increased pigmentation of Kupffer cells and/or macrophages with Perls'positive material, possibly reflecting increased iron metabolism and turnover. In dogs, also the following observations were made, a slight lowering of red cell indices, acute inflammatory cell infiltration in the lungs, decreases in heart weight, likely related to convulsions.

In these studies (rat, mice, dog), the lowest doses administered already produced adverse effects, which were sometimes quite minimal but consitent with the observations made at higher doses.

In a 21-day percutaneous test, ziram affected the liver in rabbits. This effect occurred at 300 mg/kg bw/d. No dermal reactions were observed.

In contrast to the acute experiment, repeated dosing of ziram in the rat, at doses producing systemic toxicity, did not induce behavioural and neuronal toxicity. The toxicological significance of the moderate inhibition of brain NTE is not clear. Neurotoxicity, more especially OPIDN - organophosphate induced delayed neuropathy- has been linked to profound NTE inhibition , accompanied by ageing of the agent-enzyme complex.

Long- term, repeated dose toxicity :
As in the short-term repeated dose experiments, long-term administration of ziram in rats produced decreases in body weight gain, food intake, and erythrocyte parameters suggestive of anemia.

Furthermore, rats showed the following treatment-related tissue changes: haemosiderin deposits in spleen and liver, cortical degeneration of adrenals (males), increased prominence of ultimobranchial cysts in the thyroid and C-cell hyperplasia (females), degenerative/atrophic skeletal muscles (males), axonal degeneration in spinal cord and sciatic nerves, adipose replacement of exocrine pancreas (males), lipofuschin accumulation in kidneys (females), bile duct hyperplasia in the liver, and hyperplastic and erosive lesions of the nonglandular epithelium in the stomach (males). Some of these changes already appeared at the lowest dose studied, 2.5 mg/kg bw/day and, therefore, the identification of a NO(A)EL was not possible.

At 24 mg/kg bw/d, male rats showed treatment-related haemangiomata in mesenteric lymph nodes and spleen. These haemangiomata were not pronounced, did not show evidence of malignancy and were confined to this group and sex only.

Studies from the open literature confirmed the ziram-induced muscular atrophy and sciatic nerve degeneration. Moreover, at doses higher than in the controlled studies, ziram was found to produce skeletal lesions and thyroid hypertrophy. In these studies the NOAEL was 0.7 mg/kg bw/d. In contrast to the well controlled study, one of the published studies showed that ziram, containing 6.5% of thiram known to produce C-cell hyperplasia and adenomas in female rats at the dose of 8 mg/kg bw/d, induced thyroid C-cell adenomas and carcinomas in male rats, statistically significant at a dose of 22 mg/kg bw/d. This observation might form a continuum with the C-cell hyperplasia seen in the well controlled study. In a similar study conducted on F344 rats, the incidence of C-cell hyperplasia was significantly depressed in males at 74mg/kg bw/d. In this study, male and female rats, at 74mg/kg bw/d, showed enlargement of the thyroid associated with follicular hypertrophy.
The effects on the thyroid became critical in the assessment of ziram toxicity and further explanations will be needed to clear if ziram treatment may increase or decrease the proliferating lesions of C-cell in the thyroid.

In mice, ziram did not induce tumours at dose levels up to 82 mg/kg bw for 80 weeks. In all treated groups, increased incidences of centrilobular and/or generalised hepatocellular enlargement were seen. Urinary bladder epithelial hyperplasia was observed in males and females receiving 82 mg/kg bw and in males receiving 27 mg/kg bw. The NOAEL was lower than 3 mg/kg bw/d. Pulmonary tumours, found in one study, occurred in the presence of a virus

infection which makes any interpretation difficult.

On the basis of the increased incidence of haemangiomata and thyroid C-cell hyperplasia, adenomata and carcinomata, a tumorigenic effect of high doses of ziram in the rat cannot be excluded. Since the overall conclusion with regard to mutagenicity is negative, the possible tumorigenic mechanism remains unclear.

Reproductive and development toxicity :
Ziram did not affect reproductive performance in the 2-generation rat study at the doses studied. Parental toxicity was observed in the F0 and F1 generations at the dose of 25 mg/kg bw (food concentration 540 ppm), e.g., inhibition of body weight gain and reduced food intake. At the same dose neonatal toxicity was expressed, e.g., slightly reduced pup body weights (F1 and F2).

Teratogenic effects, with regard to embryofoetal development and morphology, were not observed at doses that did not also produced maternal toxicity in rats. In rabbits, maternal toxicity was observed at 7.5 mg/kg bw/d but no teratogenic effects were reported.

From open literature, ziram has a low teratogenic effect that only becomes effective at dosages that are lethal to 50% of the mothers (100 mg/kg bw/d).

B.5.10.1 Establishment of an Acceptable Daily Intake (ADI)

An ADI is proposed on the basis of long-term toxicity in rats or mice. In these studies, effects were seen at all doses, the LOAEL being 2.5 and 3 mg/kg bw/d respectively.
It is, however, probable that the NOAEL is not much lower since in a study from open literature, 1 mg/kg bw/d was a dose without adverse effect. We therefore, feel able to propose an ADI based on a LOAEL and an assessment factor of 1000.

ADI = 0.003 mg/kg bw/d

The same proposal was made by the JMPR (1996)

B.5.10.2 Establishment of an Acceptable Operator Exposure Level (AOEL)

An AOEL as an internal, systemic dose:

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

It appears from the animal experiment that a subchronic exposure to 3.2 mg/kg bw/day (lowest NOAEL, taken from the 90 day oral study in rat, satellite study of 104 week) of ziram will not result in any toxic effect. It further appears from the ADME studies that orally ingested ziram is absorbed at a level of 58-75.6% after low dose (single or repeated) within 48 h. Therefore, a correction factor of 50% was applied. An assesssment factor of 100 is proposed for the extrapolation to man.

The acceptable operator exposure level, expressed as an internal, systemic dose becomes:

AOEL short-term = 0.016 mg/kg bw/day

(The notifier proposed the 90 day dog and the 28 day rat studies with an assessment factor of 25, giving an AOEL = $\frac{3}{25}$ = 0.12 mg/kg bw/day).

An AOEL as a dermal exposure:

A 21 day dermal study exists in the rabbit, which would allow the calculation of a dermal AOEL.In this study a

NOAEL = 100 mg/kg bw/d was reported . At the LOAEL ,systemic toxic effects were reported. Using an assessment factor of 100, this gives :

AOEL dermal = 1 mg/kg bw/day

B.5.10.3 Establishment of the drinking water limit

On the basis that exposure through drinking water should not account for more than 10 % of the ADI, assuming an average consumption of 2 l of water per person par day and an average body weight of 70 kg a limit of 0.01 mg/l is proposed.

$$MAC = \frac{ADI \times bw \times P}{C} = \frac{0.003 \times 70 \times 0.1}{2} = 0.01 \text{ mg/l}$$

MAC = maximum allowable concentration.

B.5.11 Acute toxicity including irritancy and skin sensitization of the preparations (Annex IIIA 7.1)

Ziram 76 WDG (Ziram, 76.42%) used as foliar fungicide for protecting fruit trees.

B.5.11.1.a Acute oral toxicity (Annex IIIA 7.1.1.1)

- Rat, oral administration by gavage, 2000 mg/kg bw (Kynoch et al. , 1990a, task force study)

Findings:
Mortality: occurred from within 6 hours to 21 hours in male and from 6 to 70 hours in females.
Clinical signs: Pilo-erection in all rats within 5 minutes of dosing accompanied by abnormal body carriage, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities and diarrhoea . Recovery was complete by day 5 inmale and day 4 in female rats.
Body weight: reduced body weight gain for 5 male and 1 female at 500 mg/kg bw and 3 males at 800 mg/kg bw on day 8.
Necropsy: no abnormalities were found.
Conclusion:
Combined LD50 = 666 mg/kg bw
LD_{50} male = 936 mg/kg bw
LD_{50} female = 478 mg/kg bw

Guidelines:
Experimental protocol in compliance with the test B.1 of directive 92/69/ EEC.
GLP status : yes (No atttest of competent authority)
Material and methods :
5 CD rats (Crl:CD R (SD) BR VAF plus) rats / sex /dose received by gavage a single dose of of Ziram (B.n°P891006) suspended in 1% w/v aqueous methylcellulose at 500, 800 or 1260 mg/kg bw.
The study is accepted.

- Rat, oral administration by gavage, limit test, 2000 mg/kg bw (Walker , 1991, task force study)

Findings:
A range-finding study was performed at 500 and 1000 mg/kg bw in 3 female rats and at 2000 mg/kg bw in 1 male and 1 female:
Mortality: 1 female treated with 500 mg/kg bw was found dead 3 days after treatment.
Clinical signs: at 500 mg/kg bw, animals presented hunched posture, lethargy, gasping and laboured respiration and ptosis. The same signs were reported at 1000 mg/kg bw. At 2000 mg/kg bw, animals presented hunched posture, ptosis and lethargy in the male 2 to 4 days after treatment.
In the main study, at 2000 mg/kg bw:
Mortality: 2 females were found dead, 1 day after treatment.
Clinical signs: hunched posture, lethargy and ptosis. All surviving animals appeared normal 1 to 2 days after treatment. Males presented incidents of hunched posture, lethargy or ptosis up to 9 days after treatment. In 1 male, ataxia, tiptoe gait, red/brown stains around mouth, dehydration , decreased respiratory rate were noted.
All males appeared normal after 10 days.
Body weight: reduced in 2 males.
Necropsy: in animals that died, abnormal red lungs, dark liver and kidneys, and haemorrhage of the gastric mucosa were noted. Stomach and small intestine contain a white substance.
Conclusion:
LD_{50} > 2000 mg/kg bw : The study is not accepted as 2 female rats were found died at 2000 mg/kg bw.

Guidelines:
Experimental protocol in compliance with the test B.1- limit test - of directive 92/69/ EEC.
GLP status: yes
Material and methods :
5 Sprague-Dawley rats /sex received by gavage a single dose of of Ziram (B.n°1407AA) suspended in distilled water at 2000 mg/kg bw.

B.5.11.2.a Acute percutaneous toxicity (Annex IIIA 7.1.2)

- Rabbit, dermal application, limit test, 2000 mg/kg bw (Kynoch , 1990b, Task force study)

Findings :
Mortality: no dead animals.
Clinical observations : no signs of systemic toxicity.
Body weight: 1 female (day 8) and 1 male and 1 female (day 15) showed a slight body weight loss
Gross pathology : all findings were normal.
Conclusions :
LD_{50} > 2000 mg/kg.

Guidelines :
Protocol not fully in compliance with method B.3 of Directive 92/69/EEC (limit test) .
Deviation from the official protocol : no data about necropsy or clinical signs.
GLP status: the study is GLP (no attest of the competent authority).
Material and Methods :
5 Male and 5 female New Zealand white rabbits were exposed to 2000 mg/kg bw Ziram 76% WG moistened with distalled water (B.n° 891006) under occluded dressing for 24 h.

B.5.11.3.a Acute inhalation toxicity to rats (Annex IIIA 7.1.3)

not relevant for WG formulation

B.5.11.4.a Skin irritation (Annex IIIA 7.1.4)

- Rabbit, skin irritation, 0.5 g (Liggett, 1990a, Task force study)

Findings:
Dermal observations:
Evaluation of the data, according to the EU methodology, gave the following results:

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

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

Conclusions :
Ziram 76% WG is not a skin irritant.

Guidelines :
Protocol fully in compliance with method B.4 of Directive 92/69/EEC.
GLP status: the study is GLP (no attest of the competent authority).
Material and Methods :
0.5 g Ziram 76% WG (B.n° P891006) in 0.5 ml distilled water was applied to the clipped intact skin of 6 New Zealand white rabbits for 4 hours under a semi-occlusive dressing.

B.5.11.5.a Eye irritation (Annex IIIA 7.1.5)

- Rabbit , eye instillation, 0.1 ml (Liggett, 1990b, Task force study)

Findings:
24 hours after instillation, an opacity covering the entire corneal surface was present. Iridial inflammation had developed by the 24 hour reading. A diffuse crimson-red coloration of the conjunctivae accompanied by considerable swelling with the eyelids half-closed and a copious discharge were observed 24 h following instillation.

Necrosis of the nictitating membrane was also present . Due to the severity of the response only one animal was exposed to Ziram 76 WG and the study was terminated on a human grounds 24 h after instillation.
Evaluation of the data, according to the EU methodology, gave the following results:

Table B.5.11.5.a-1 : Eye irritation properties of Ziram 76 WG

Region of the eye	1 hour	1 day
cornea	0	2
iris	0	1
conjunctiva : redness	2	2*
conjunctiva : chemosis	2	3

* : necrosis of nictitating membrane

Conclusions :
Ocular lesions are significant . Ziram is irritating to eyes (R 41)

Guidelines:
Protocol in compliance with method B.5 of Directive 92/69/EEC.
GLP status: the study is GLP (no attest of the competent authority).
Material and Methods :
0.1 ml (60 mg) Ziram 76% WG (B.n° P891006) was placed into the conjunctival sac of the left eye of each New Zealand white rabbit.

B.5.11.6.a Skin sensitization (Annex IIIA 7.1.6)

- Skin sensitizing potential in guinea pigs (Split Adjuvant Test) (Daamen, 1989, task force study)

Findings :
No signs of systemic toxicity were observed during the primary irritation experiments.
Fourteen experimental animals showed skin irritation after the last occluded epicutaneous induction exposure. Four animals showed a positive reaction in response to the 25% concentration and four animals in response to the 10% and the 5% concentration. However, over the range of test article concentrations tested, five animals showed a positive response. The reactions were characterised with moderate and confluent redness, swelling and scaliness.

Conclusion :
According to the experimental results reported in the study, Ziram 76 WG is a moderate sensitizer (25% positive animals) but needs no classification

Guidelines:
The test to be carried out in accordance with dir. 92/69/EEC method B.6(84/449/EEC)is the method Maximization test which is the preferred test. The Split Adjuvant Test was used .
GLP status : yes (no attest of competent authority)
Material and methods :
20 female Guinea-pigs (Dunkin Hartley) were used for the experimental group.
Induction: On day 1 a window dressing was prepared; on day 3, 0.2 ml Ziram 76 WG, 50% in corn oil (lot n°.P891006) was applied and on day 5 , Freund Complete adjuvant was injected intradermally and this was followed by a further application of 0.2 ml test substance 50% (w/w). On day 8 , the application site was clipped and again 0.2 ml of 50% (w/w) test substance was applied. On day 10, all wrappings and test substance were removed and the skin reaction was observed. Challenge two weeks after and 3 test substance concentrations were applied (25%; 10%; 5% or corn oil only) on the shaved areas of the same animal. Dressings were removed after 24 h.
10 animals served as control group and 5 animals were used for the primary irritation test.
The study is accepted.

Summary of acute toxicity of Ziram 76 WG:

Type of test	Result	Batch n°.	Reference	Classification
LD_{50} oral rat male female	= 936 mg/kg bw = 478 mg/kg bw	B.n°P891006	Kynoch et al. , 1990, task force study	Xn, R 22
LD_{50} dermal rat	LD_{50} > 2000 mg/kg bw	B.n° P891006	Kynoch , 1990, Task force study	
LC_{50} rat	not relevant for WG formulation			
Skin irritation rabbit 4 h	not a skin irritant	B.n° P891006	Liggett, 1990a, Task force study	
Eye irritation rabbit	severe irritating to eyes	B.n° P891006	Liggett, 1990b, Task force study	Xi, R 41
Skin sensitization guinea pig Split Adjuvant test	moderate sensitizer	B.n° P891006	Daamen, 1989, task force study	

B.5.11.7.a Additional studies for combinations of plant protection products (Tests as at points 7.1.1 to 7.1.6) (Annex IIIA 7.1.7)

No data, not necessary.

B.5.12.1.a Dermal absorption, *in vivo* in the rat (Annex IIIA 7.3)

Dermal toxicity of ziram formulation is low (LD_{50} > 2000 mg/kg bw).

A study was conducted to evaluate the extent of absorption of radiolabeled ziram following dermal administration of 1.07, 11.9 or 90.6 mg/animal to male rats. Animals were sacrificed at 0.5, 1, 2, 4, 10 and 24 h after dosing.

Table B.5.12.1a-1: Dermal absorption, *in vivo* in the rat

dose mg/animal	mean absorbed dose (%)				total (mg)
	skin	urine, carcass, blood	nonaccount	total	
1.07	5.02	0.306	24.4	29.72	0.318
11.9	5.91	0.08	26.2	32.19	3.83
90.6	1.17	<0.01	3.37	4.55	4.14

After low, medium or high dose application, absorption of radioactivity was 29.8, 32.2 or 4.5% respectively ; 76.2%, 68.7% and 99.9% of radioactivity was recovered in samples of skin wash, skin cell cover and skin enclosure. Mean adsorbed radioactivity in the skin was 5.02, 5.91 or 1.17% for a low , medium or high dose and suggested that skin adsorption/absorption was non linear to the dose applied.
In this study, nonaccountable radioactivity was high at low and medium dose representing respectively 19 and 26% of the administered dose and may have been eliminated in the expired air.
(The dose of 91 mg/animal represents a dose of approximately 273 mg/kg bw).

B.5.13.a Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

No data, not necessary.

B.5.14.a Exposure data (Annex IIIA 7.2)

B.5.14.1.a Estimation of operator exposure (Annex IIIA 7.2.1.1)

Ziram WG formulations are mainly applied in orchards (pomefruit, stonefruit, almond). The application rates are in the range 1.18-3.04 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, the type of material used and the size of trees. The operator exposure was estimated by the UK POEM and the German model for the following typical scenario :

Orchard treatment (cherry, plum, peach) , 3.04 kg/ha, 304 g a.s./hl.

UK POEM model	German model
Use rate : 3.04 kg a.s./ha	Use rate : 3.04 kg a.s./ha
Number ha treated/day : 30 ha/day	Number ha treated/day : 8 ha/day
Method of use: vehicle air assisted broadcast > 400 l/ha	Method of use: high crop tractor mounted
Body weight : 60 kg	Body weight : 70 kg
AOELsys : 0.016 mg/kg bw/d	Dtol : 70 (based on AOELdermal = 1 mg/kg bw/d)
Dermal absorption: 5% during M/L 30% during A	Itol : 1.12 (based on AOELsys = 0.016 mg/kg bw/d)

Expected operator exposure :

Table B..5.14.1- 1 : Estimated Operator exposure (mg/person/day)according to the UK POEM model

Application method: tractor drawn air assisted orchard sprayer	**Dermal absorbed dose** (mg/person/day)		**Inhalation exposure** (absorbed dose)(mg/person/day)	**Total absorbed dose** (mg/person/day)
	M/L	A	A	
No protection	91.2	110.53	0.912	202.64
Gloves during M/L and A	0.912	77.7	0.912	79.52

Table B..5.14.1- 2 : Estimated Operator exposure (mg/person/day) according to the German model

Application method: tractor drawn air assisted orchard sprayer	**Dermal exposure** (mg/person/day)			**Inhalation exposure** (mg/person/day)			**Total** (mg/person/day)
	M/L	A	Total	M/L	A	Total	
No protection	48.64	279.7	328.3	0.194	0.438	0.632	325.97
Gloves during M/L and A = standard protective garment and strudy foot wear	0.486	41.02	41.50	0.194	0.438	0.632	42.13

Comparison of estimated and tolerable exposure:

Table B..5.14.1- 3 : Exposure as a proportion of the AOEL - UK POEM model

Application method: tractor drawn air assisted orchard sprayer	**Total systemic exposure- 60 kg person (mg/kg bw/d)**		**% AOEL**	
	No PPE	PPE	No PPE	PPE
	3.377	1.325	21109	8250

Table B..5.14.1- 4 : Total degree of exposure E - German model

Application method: tractor drawn air assisted orchard sprayer	**Total (external) exposure- 70 kg person (mg/kg bw/d)**		**E**	
	No PPE	PPE	no PPE	PPE
	4.656	0.601	5.22	1.15

Conclusion :
The operator exposure exceeds the values (% AOEL, degree of exposure E) even when the operator wears gloves during mixing/loading and spraying as well as standard protective garment and sturdy footwear during the application.

According to the UK POEM model, dermal exposure to ziram, as an external dose is 36.54 mg/kg bw/day without PPE. When PPE are worn, dermal exposure to ziram is reduced to 4.6 mg/kg bw/day. These values exceed the dermal AOEL Absorbed dose via inhalation reaches 0.0152 mg/kg bw/d and is lower than the systemic AOEL.

B.5.14.2a Measurement of operator exposure (Annex IIIA 7.2.1.2)

Exposure of mixer/loaders and applicators to Ziram 76 WGD fungicide applied by ground equipment in Orchard crops in California, 1989.
Apple orchards were sprayed using typical airblast sprayers pulled by open-cab tractors or tractors with no cabs; sprayers were loaded using typical open-pour loading equipment. Ziram 76 WDG was diluted into water for use on apples at the use rate of 10.2 kg a.s./ha. Three mixer/loaders and three applicators were monitored for five replicates each during the study. Each replicate consisted of mixing/loading or applying one tankload of Ziram 76 WDG under the following diverse conditions:
3 Mixer/loader and 3 applicators were controlled in this study.

Table B.5.14.2a-1 : Measurement of operator exposure.

Site	Replicate	Spray concentration (g a.s./hl)	application rate kg a.s./ha	Equipment	Environmental conditions
Site1: Smith Orchard 1 mixer/loader of 100 kg 1 applicator of 109 kg	1	380	5.5	airblast sprayer with 1890 l capacity tank and 7 open D8-45-nozzles per side. 4-Wheel drive tractor with no cab driven at a speed of 1.7 km/h	temp : 34-44°C relative humidity: 31-67%
	2	380	5.38		
	3	380	7.17		
	4	550	10.2		
	5	550	10.2		
Site 2: Cannella farms 1 mixer/loader of 64 kg 1 applicator of 82 kg	6-10	400	10.08	airblast sprayer with 2000 l capacity tank and 14 -5 disks with 2 hole spinners/side operating at 125 psi.an open cab tractor used	temp : 37-47°C relative humidity:18-48%
Site 3 : Harper apples orchard 1 mixer/loader of 83 kg 1 applicator of 61 kg	11-15	4.13	10.32	airblast sprayer with 1892 l capacity steel tank and 13D6-46 and 8 D6 (no spinners) nozzles per side operating at 120psi. Tractor with no cab.	temp : 35-45°C relative humidity: 35-58%

The target rate of application was 10.2 kg a.s./ha (the maximum labelled rate in the USA). In replicates 1-3, the pump did not maintain the pressure setting determined from calibrations with water; the application rates were therefore lower than the nominal, but typical for ziram use.

Dermal exposure exclusive of the face, throat, and hands, was estimated from residues on 100% cotton suits worn underneath worker's conventional clothing. Workers wore long-sleeved coveralls, shoes or boots, respirator, goggles, gloves and a cap. Exposure of the face and throat was measured from residues on gauze surgical sponges which were used to swab areas of approximately 15 cm^2 each on the forehead, left cheek, right cheek and throat. Exposure of gloved and bare hands was measured by bag rinses with distilled water followed by a solution of 0.5% soap .

Inhalation exposure was estimated by measuring residues collected in a 10% aqueous EDTA solution in glass midget impingers placed in the worker's breathing zone and attached to personal air pumps.
Measured residues were adjusted fot the mean recovery of ziram from monitoring media fortified in the field. The fortification solution was prepared by diluting the formulation into water at approximately the application dilution rate.
The geometric mean rates of dermal and inhalation exposure from the 15 replicates were standardized to µg/kg bw/kg a.s. handled:

Table B.5.14.2a-2 : Measurement of operator exposure to ziram (Meikle, 1991, Task force study)

geometric mean rates of exposure (µg/kg bw/kg a.s.)		
	mixer/loaders (x ± sd)	**applicators (x ± sd)**
dermal exposure		
working with gloved hands		
hygiene	0.59 ± 2.1	0.38± 2.8
no hygiene	0.63 ± 2.1	0.41 ± 2.6
working with bare hands		
hygiene	1.75 ± 2.4	0.72 ± 2.6
no hygiene	3.79 ± 2.2	1.17± 2.7
inhalation exposure	0.20 ± 2.17	0.05± 3.2

From these measurements, the RMS calculated the absorbed dose to compare the results with the AOEL assuming a dermal absorbed dose of 30% for the diluted formulation and of 5% for the WG based on the dermal absorption study in rats *in vivo*.
The application conditions of the study, 380-550 g a.s./hl are similar to the intended uses of ziram in the EU (75-304-532 g a.s./hl). The difference of application rates/ha play a minor role because the extrapolation to the EU intended uses is based on an exposure expressed in µg/kg bw/kg a.s. applied.
The total absorbed dose was calculated for 1 kg a.s. (table B.5.14.2a-3) and for 3.04kg a.s. and 30 ha (table B.5.14.2a-4). The latter results were compared to the AOELsys.

Table B.5.14.2a-3: Total absorbed dose (µg/kg bw/applied kg a.s.) calculated according to the results of actual exposure

	Dermal exposure (µg/kg bw/kg a.s.)		**Inhalation exposure** µg/kg bw/(kg a.s.)	**Dermal absorbed dose** (µg/kg bw/kg a.s.)		**Total absorbed dose** (µg/kg bw/kg a.s.)
Protective equipment	Mixing/ loading	Application	Mixing/loading+ Application	Mixing/ loading (5%)	Application (30%)	
w/o PPE	2.77 (*)	0.945 (*)	0.2 + 0.05 = 0.25	0.138	0.283	0.671
gloved hands	0.6 (*)	0.4 (*)	0.2 + 0.05 = 0.25	0.03	0.12	0.4

(*) : mean of exposure with and without hygiene

Table B.5.14.2a-4 : Total absorbed dose and % of the AOEL for an application of 3.04 kg ziram /ha, 30 ha/day.

Protective equipment	**Dermal absorbed dose (µg/kg bw/day)**	**Absorbed dose by inhalation (µg/kg bw/day)**	**Total (µg/kg bw/day)**	**% AOEL**
w/o PPE	38.4	22.8	61.2	382
gloved hands	13.7	22.8	36.5	228
gloved hands + half mask	13.7	22.8 x 0.02 = 0.456	14.1	88

Conclusion:

Exposure is acceptable when gloves and a half-mask are worn.

Comment :
Exposure parameters from the actual US orchard monitoring study were inserted into the UK POEM model to compare theorical and actual results in USA conditions . From the table B.5.14.2a-5, it appears that the model overestimates the dermal exposure during mixing/loading and application. Inhalation figures were comparable.

Table B.5.14.2a-5 : Comparison of exposure as calculated according to the UK POEM model and the actual exposure in the orchard crop, 5.09 kg a.s./ha, 30 ha/day.

	External exposure according to UK POEM			**External exposure according to actual measurement**		
	Dermal exposure (mg/person/day)		**inhalation** (mg/person /day)	**Dermal exposure*** (mg/person/day)		**inhalation*** (mg/person /day)
	M/L	**A**	**A**	**M/L**	**A**	**ML+A**
w/o PPE	6110	617.1	1.527	25.13	8.5	2.26

* Calculation is based on dermal or inhalation exposure (table B.5.14.2a-3) in µg/kg bw/kg a.s. x 60 (kg bw) x 5.04 kg (a.s./ha) x 30 (ha).

B.5.14.3a Estimation of bystander exposure (Annex IIIA 7.2.2)

Considering a spray drift of 4.5%, the exposure of a bystander is as follows :
E_{dermal} = (368 mg/day x 0.045) /60 = 0.276 mg/kg bw < $AOEL_{dermal}$
$E_{inhalation}$ = (0.912 x 0.045)/60 = 0.00068 mg/kg bw < $AOEL_{sys}$.

The risk for the bystander is acceptable.

B.5.14.4a Estimation of worker exposure (Annex IIIA 7.2.3.1)

This point should be addressed by the notifier.

B.5.14.5a Measurement of worker exposure (Annex IIIA 7.2.3.2)

no data.

B.5.15 References relied on

Toxicology and metabolism (Annex IIA 5; Annex IIIA 7)

Toxicology and metabolism of the active substance (Annex IIA 5)

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
IIA5.6.2	Barker L.	Ziram : oral (gavage) range findings study in the pregnant rabbit Generated by : Hazleton Laboratories Europe Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 4588-508/1 Date : 09/1985	no	unpublished protected
IIA5.6.2	Barker L.	Ziram : oral (gavage) teratology study in the rabbit Generated by : Hazleton Laboratories Europe Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 4913-508/2 Date : 07/1986	yes	unpublished -
IIA5.2.3	Blagden S.M.	Ziram technical. Acute inhalation toxicity study. Four hour exposure (nose only) in the rat Generated by : Safepharm Laboratories Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 378/2 Date : 05/11/1991	yes	unpublished protected
IIA 5.1.3	Bodden R.M.	Ziram: Nature of the residues in Lactating goats Generated by : Hazleton Laboratories America, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : HLA 6225-101 Date : 16/06/1993	yes	unpublished protected
IIA 5.4.1	Brooker P.C. Akhurst L.C.	Analysis of metaphase chromosomes obtained from CHO cells cultured *in vitro* and treated with Ziram Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 7/89675 Date : 05/09/1989	yes	unpublished -
IIA 5.3.1	Chambers P.R. Cary R.J. Gibson W.A. Gopinath C.	Ziram palatability study in rats for 4 weeks Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 2/89719 Date : 06/05/1992 (a)	yes	unpublished protected
IIA 5.3.1	Chambers P.R. Cary R.J. Gibson W.A. Gopinath C.	Ziram palatability study in mice for 4 weeks Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 3/89720 Date : 06/05/1992 (b)	yes	unpublished protected

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
IIA 5.1.1	Cheng Th.	Metabolism of Ziram in Rats Generated by : Hazleton Laboratories America, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : HLA 6225-106 Date : 01/02/1991 Annex : Summary Report : Pilot Metabolism study of Ziram in rats. HLA study 6225-104.	yes	unpublished -
IIA 5.1.1	Cheng Th.	Dermal absorption of ^{14}C-Ziram in male rats Generated by : Hazleton Laboratories America, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : HLA 6225-107 Date : 01/02/1991 Annex : Summary Report : Pilot Dermal Absorption study of ^{14}C-Ziram in male rats. HLA 6225-105.	yes	unpublished protected
IIA5.4.2	Ciliutti, P and Brightwell, J.	Ziram : micronucleus test. Generated by : Research Toxicology Centre S.P.A., Pomezia Italy Submitted by : Bakelite Company File No. : 4400/T/M/00895 Date : 12/04/1995	yes	unpublished -
IIA 5.4	Crebelli, R., Zijno,A., Conti,L.,Croch, B., Leopardi,P., Marcon,F., Renzi,L. and Carere, A.	Further *in vitro* and *in vivo* mutagenicity assays with Thiram and ziram fungicides: bacterial reversion assays and mouse micronucleus test. Reference: Teratogenesis, Carcinogenesis and Carcinogenesis, 12, 97-112 Date: 1992	yes	unpublished -
IIA5.2.6	Daamen P.A.M.	Assessment of the skin sensitisation potential of Ziram Technical in the albino guinea pig (Split Adjuvant Test) Generated by : RCC Notox B.V. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : RCC NOTOX 0878/1096 Date : June 1988	yes	unpublished -
IIA5.3.1	Dickhaus S. Heisler E.	Report of a subacute toxicity study of the substance "Ziram" (technical) for 4 weeks in the rat Generated by : Pharmatox, Forschung und Beratung GmbH Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 2-4-132-80 Date : 09/1980	no	unpublished protected
IIA 5.4	Donner,M.	Use of *Drosophila* as indicator of risk chemicals in rubber factory Reference:SGF publications 57,47-53	no	published -

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference Date:1981	GLP GEP	Published Protected
IIA 5.4	Donner,M., Husgafvel-Pursiainen,K., Jenssen,D. and Rannug,A.	Mutagenicity of rubber additives and curing fumes. Reference: Scand.J.Work. Environ.Health,9 27-37 Date:1983	yes	unpublished -
IIA5.3.3	Edwards J.A. Baldrick P. Gibson W.A. Crook D. Offer J.M. Gopinath C.	Twenty-one day dermal toxicity study in rabbits with Ziram Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 4/89689 Date : 02/11/1989	yes	unpublished -
IIA5.5	Enomoto, A., Harada, T., Maita, K., Shirasu, Y.	Epiphyseal lesions of the femur and tibia in rats following oral chronic administration of zinc dimethyldithiocarbamate (ziram). Toxicology, 54, 45-58, 1989	No	published -
IIA5.4.2	Falezza,A, and Brightwell, J.	Ziram : Unscheduled DNA synthesis (UDS) in primary rat hepatocytes after *in vivo* treatment Generated by : Research Toxicology Centre S.P.A., Pomezia Italy Submitted by : Bakelite Company File No. : 4531/M/01395 Date : 12/04/1995	yes	unpublished -
IIA 5.4	Foureman, p., Mason, J.M., Valencia, R., Zimmering, S.	Chemical mutagenesis testing in Drosophila.X. Results of 70 coded chemicals tested for the National Toxicology program. Reference: Environ. Mol.Muta. 23, 208-227. Date: 1994	no	published -
IIA5.6.2	Giavini, E., Vismar, C., Broccia, M.L.	Pre- and Postimplantation embryotoxic effects of zinc dimethyldithiocarbamate (ziram) in the rat. Reference : Ecotox. Environ. Safety; 7, 531-537, 1983	no	published -
IIA5.5	Gopinath, C.	Comparative endocrine carcinogenesis Conference on endocrine toxicology, Presented by Henry Stewart Conference Studies. London, december 3, 1997	No	unpublished -
IIA5.4.2	Gulati, D.K., Witt,K., Anderson, B., Zeiger, E. and Shelby, M.	Chromosome aberration and sister chromatid exchange tests in chinese hamster ovary cells in vitro III.: Results with 27 chemicals. Reference: Environ.Mol.Muta. , 13, 133-193 Date: 1989	yes	unpublished -
IIA5.2.4	Haynes,G. and Brightwell, J.	Ziram : acute dermal irritation study in the rabbit Generated by : Research Toxicology Centre S.P.A., Pomezia Italy	yes	unpublished -

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
		Submitted by : Bakelite Company File No. : 4397/T/220/94 Date : 13/04/1995a		
IIA5.2.5	Haynes,G. and Brightwell, J.	Ziram : acute eye irritation study in the rabbit Generated by : Research Toxicology Centre S.P.A., Pomezia Italy Submitted by : Bakelite Company File No. : 4398/T/255/94 Date : 13/04/1995b	yes	unpublished -
IIA5.2.6	Haynes,G. and Brightwell, J.	Ziram : delayed dermal sensitisation study in the guinea-pig Generated by : Research Toxicology Centre S.P.A., Pomezia Italy Submitted by : Bakelite Company File No. : 4399/T/031/95 Date : 13/04/1995c	yes	unpublished -
IIA 5.4	Haworth, S., Lawlor,T., Mortelmans, K., Speck, W. and Zeiger,E.	Salmonella Mutagenicity test results for 250 chemicals. Reference : Environm. Mutagenesis, suppl. 1, 3-142 Date: 1983	yes	unpublished -
IIA 5.4	Hedenstedt, A., Rannug, U., Ramel, C., Wachtmeister, C.A.	Mutagenicity and metabolism studies on 12 thiuram and dithiocarbamate cmopounds used as accelerators in the swedish rubber industry. Reference: Mutation Res., 68, 313-325 Date: 1979	yes	unpublished -
IIA 5.4	Hemavathi,E. and Rahiman,M.	Toxicological effects of ziram, Thiram and dithane M-45 by sperm shape abnormalities in mice. Reference: J. Toxicol.Environ.Health, 38, 393-398. Date:1993	no	published -
IIA5.4	Hemavathy, K.C. and Krishnamurty, N.B.	Cytogenetic effects of Cuman L , a dithiocarbamate fungicide Reference: Mutation Res., 208, 57-60 Date: 1988	no	published -
IIA5.4	Hemavathy, K.C. and Krishnamurty, N.B.	Genotoxicity studies with Cuman L in Drosophila melanogaster Reference: Environ.Mol.Muta. 14, 252-253 Date: 1989	no	published -
IIA5.2.3	Jackson G.C., Hardy C.J.	Ziram Technical. Acute inhalation toxicity in rats, 4-hour exposure Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB. S.A., ELF ATOCHEM Company File No. : UCB 314/89684	yes	unpublished protected

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
		Date : 4/12/89		
IIA5.4.1	Jones E. Cook P.G.S. Gant R.A. Kitching J.	Ziram technical : bacterial mutation assay Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 25/891914 Date : 22/08/1990	yes	unpublished -
IIA5.4	Kada, T., Hirano, K., Shirasu, Y.	Screening of environmental chemical mutagens by the Rec-Assay system with Bacillus subtilis Reference: in :Chemical mutagens- Principle and methods for their detection, Eds: F.de Serres and A.Hollaender vol 6, 1980, pp 149-173	no	published -
IIA5.2.3	Kieran, P.C.	Ziram acute inhalation toxicity study in rats Generated by : Inveresk Research International Ltd. Submitted by : Bakelite Company File No. :IRI report n°.12101 Date : 05/04/1985	no	unpublished -
IIA5.7	Lamb I.C.	<u>An acute neurotoxicity study of Ziram in rats</u> Generated by : WIL Research Laboratories, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : WIL - 223001 Date : 1994, Final Draft	yes	unpublished protected
IIA5.7	Lamb I.C.	<u>A subchronic (13-week) neurotoxicity study of Ziram in rats</u> Generated by : WIL Research Laboratories, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : WIL - 223004 Date : 17/09/1993 Audited Draft	yes	unpublished protected
IIA5.2.1	Liggett M.P. Allen S.A.	Acute oral toxicity to rats of Ziram Generated by : Huntingdon Research Centre Ltd Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 89690D/UCB 315/AC Date : 30/11/1989	yes	unpublished -
IIA5.2.2	Liggett M.P. Allen S.A.	Acute dermal toxicity to rabbits with Ziram Generated by : Huntingdon Research Centre Ltd Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 89338D/UCB 316/AC Date : 30/11/1989	yes	unpublished -
IIA5.2.4	Liggett M.P. McRae L.A.	Skin irritation to rabbits with Ziram (Technical) Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 90500D/UCB 317/SE Date : 14/08/1990	yes	unpublished -
IIA5.2.5	Liggett M.P.	Eye irritation to rabbits with Ziram (Technical)	yes	unpublished

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	McRae L.A.	Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 90499D/UCB 318/SE Date : 14/08/1990		-
IIA5.9.4	Liesivuori J. Savolainen K.	Chapter 5 : Dithiocarbamates.Toxicology 91 (1994) 37-42	yes	published -
IIA5.9.1	Mahy I.	Letter concerning health effects of Ziram	yes	published -
IIA 5.5	Maita K., Enomoto A., Nakashima N., Yoshida T., Sugimoto K., Kuwahara M. and Harada T.	Chronic toxicity studies with ziram in F3444 rats and Beagle dogs J. Pesticide Sci., 22, 193-207, 1997	no	published -
IIA5.3.1	McLean T.A. Horner S.A. Buist D.P.	Ziram, preliminary dietary toxicity study in Beagle dogs (repeated daily dosage for 4 weeks) Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 1-G/89637 Date : 30/04/1992a	yes	unpublished protected
IIA5.3.2	McLean T.A. Horner S.A. Buist D.P. Crook D. Read R.M. Gopinath C.	Ziram toxicity study on beagle dogs (repeated daily dosage for 13 weeks) Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 8-G/901813 Date : 05/05/1992b	yes	unpublished -
IIA 5.4	McGregor, D.M., Brown, A., Cattanach, P., Edwards, I., McBride, D.,Riach, C., Caspary, W.	Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay: III. 72 Coded Chemicals. Reference: Environ.Mol.Muta., 12, 85-154 date: 1988	yes	unpublished -
IIA 5.4	Mosesso,P., Turchi,G. Cinelli,S., Di Chiara,D., Fiore,M. and Palitti,F.	Clastogenic effects of the dithiocarbamate fungicides Thiram and ziram in chinese hamster cell lines cultured *in vitro*. Reference: Teratogenesis, Mutagenesis and Carcinogenesis, 14, 145-155 Date:1994	yes	unpublished -
IIA5.6.1	Nemec, M. D.	A dietary two-generation reproduction and developmental neurotoxicity study of ziram in rats. Generated by : Wil research laboratories, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : WIL223003	yes	unpublished protected

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference Date : 30/0/1996	GLP GEP	Published Protected
IIA5.5	NTP/NIEHS study	TR-238 Carcinogenesis bioassay of ziram (CAS n°. 137-30-4) in F344/N rats and B6C3F1 mice (Feed study) Environ.Health Perspect. 101, 1983, 130-131	No	published -
IIA5.9.5	Pestline	Pestline : Material Safety Data Sheets for Pesticides and Related Chemicals (Volume II) : Zinc dimethylditiocarbamate	yes	published -
IIA5.5	Powell L.A.J. Bottomley S.M. Crook D. Majeed S.K. Gopinath C. Gibson W.A. Anderson A.	Ziram (Technical). Potential oncogenicity to mice by repeated dietary administration for 80 weeks Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 12/932311 Date : 19/08/1994b	yes	unpublished protected
IIA5.5	Powell L.A.J. Battomley S.M. Crook D. Gregson R.L. Offer J.M. Gibson W.A. Anderson A.	Ziram (Technical). Combined chronic toxicity and oncogenicity of Ziram (technical) administered in the diet to rats Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 9/942098 Date : 27/09/1994a	yes	unpublished protected
IIA5.3.2	Powell L.A.J. Gopinath C. Gibson W.A. Anderson A.	Ziram preliminary toxicity to mice by dietary administration for 13 weeks Generated by : Huntingdon Research Centre Ltd Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 11/901841 Date : 05/11/1993	yes	unpublished protected
IIA5.3.2	Powell L.A.J. Crook D. Gregson R. Gopinath C. Gibson W.A. Anderson A.	Ziram preliminary toxicity to rats by dietary administration for 13 weeks Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 5/901840 Date : 19/08/1992	yes	unpublished -
IIA5.4.1	Proudlock R.J.	Auto-radiographic assessment of DNA repair after *in vitro* exposure of rat hepatocytes to Ziram Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 6/89820 Date : 12/09/1989	yes	unpublished -
IIA5.4.2	Proudlock R.J. Taylor K.	Ziram (Technical). Peripheral blood micronucleus test in the mouse after 89 days dietary exposure Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 27/920513	yes	unpublished protected

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
		Date : 14/9/1992		
IIA5.6.1	Ritter D.L. Bowie M.D.	Three generation reproduction study of Vancide MZ-96 (lot No. GP-26-24M) Zinc dimethyldithiocarbamate in Sprague-Dawley rats Generated by : Cannon Laboratories, Inc. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 6E2374 Date : 01/05/1979 - revised : 03/03/1982	no	unpublished -
IIA5.4	Shirasu, Y., Moriya, M., Kato, K., Furuhashi, A. and kada, T.	Mutagenicity screening of pesticides in the microbial system. Reference: Mutation Res., 40, 19-30 Date: 1976	no	published -
IIA 5.4	Siebert,D., Zimmerman, F.K. and Lemperle,E.	Genetic effects of fungicides. Reference : Mutation Res., 10, 533-543 date: 1970	no	published -
IIA5.5	Smith T.G. Buist D.P. Crook D. Morrow J. Gopinath C.	<u>Ziram toxicity to dogs by repeated dietary administration for 52 weeks</u> Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 10/920533 Date : 08/06/1993	yes	unpublished protected
IIA5.4	Tripathy, N.K., Majhi, B., Dey, L. and Das, C.	Genotoxicity of ziram established through wing, eye and female ger-line mosaic assays and the sex-linked recessive lethal test in Drosophila melanogaster Reference: Mutation Res., 224, 161-169 Date: 1989	no	published -
IIA5.9.2	Savolainen, K.	Tiuramer och dimethyldithiokarbamater. Arbete och Hlsa, 26, 1990, 35 p.	No	published -
IIA5.6.2	Smith J.A. Bryson A.M. John D.M. Anderson A.	A study on the effect of Ziram on pregnancy of the rat Part II : a preliminary study of the effect of Ziram on pregnancy of the rat Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 15/891371 Date : 11/06/1990	yes	unpublished -
IIA5.6.2	Smith K.A. Bryson A.M. John D.M. Anderson A.	A study on the effect of Ziram on pregnancy of the rat. Part I. Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : ZIR 24/891371	yes	unpublished -

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
		Date : 11/06/1990		
IIA5.4.2	Völkner W.	Chromosome aberration assay in bone marrow cells of the mouse with Ziram technical (UCB provenience) Generated by : Cytotest Cell Research GmbH & Co. KG Submitted by : UCB S.A., ELF ATOCHEM Company File No. : CCR 254812 Date : 26/02/1992 (a)	yes	unpublished -
IIA5.4.3	Völkner W.	Mouse germ-cell cytogenetic assay with Ziram technical (UCB provenience) Generated by : Cytotest Cell Research GmbH & Co. KG Submitted by : UCB S.A., ELF ATOCHEM Company File No. : CCR 254834 Date : 17/09/1992 (b)	yes	unpublished protected
IIA 5.4	Wyss-Benz, M.	14C-Ziram plant metabolism study in field grown apple Generated by : RCC umweltchemie AG Submitted by Ziram task force Report/file No. RCC project 350673 Date of report: 1994	yes	published protected

Toxicology and metabolism of the formulation ZIRAM 76WG (Annex III A 7)

Annex point(s) 91/414/EEC	Author(s)	Title Generated by (company or organisation), Submitted by (company(ies) or organisation), Report/file No. of submitting company Date of report for publication: reference	GLP GEP	Published Protected
IIIA7.1.5	Daamen P.A.M.	Skin sensitization to ZIRAM 76% WG in the albino guinea pig (Split Adjuvant Test) Generated by : RCC NOTOX B.V. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : RCC NOTOX 017280 Date : 01/12/1989	yes	unpublished protected
IIIA7.1.1	Kynoch S.R. Denton S.M. Healing G.	Acute oral toxicity to rats of Ziram 76% WG Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 9074D/UCB 326/AC Date : 21/03/1990a	yes	unpublished protected
IIIA7.1.2	Kynoch S.R.	Acute dermal toxicity to rabbits of Ziram 76% WG Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 9053D/UCB 327/AC Date : 25/07/1990b	yes	unpublished protected
IIIA7.1.4	Liggett M.P.	Skin irritation to rabbits with Ziram 76% WG Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 9068D/UCB 328/SE Date : 15/03/1990a	yes	unpublished protected
IIIA7.1.4	Liggett M.P.	Eye irritation to rabbits with Ziram 76% WG Generated by : Huntingdon Research Centre Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 9069D/UCB 329/SE Date : 14/03/1990b	yes	unpublished protected
IIIA7.2.1.2	Meikle Susan B.	Exposure of mixer/loaders and applicators to Ziram® 76 WDG fungicide applied by ground equipment in California, 1989 Generated by: ORIUS Associates Inc. Submitted by: UCB S.A., ELF ATOCHEM Company File No.: 27-ZIR/91050 Date : 10,06,1991.	yes	unpublished protected
IIIA7.1.1	Walker D.J.	Ziram 76 WG : Acute oral toxicity (Limit test) in the rat Generated by : Safepharm Laboratories Ltd. Submitted by : UCB S.A., ELF ATOCHEM Company File No. : 378/1 Date : July 1991	yes	unpublished protected

ANNEX B

Ziram

Appendix C : Estimation of the operator exposure

Estimation of the operator exposure

1 UK model - tractor drawn air assisted orchard sprayer - 304 g a.s./hl without PPE

2 UK model - tractor drawn air assisted orchard sprayer - 304 g a.s./hl - with PPE

3 UK model - tractor drawn air assisted orchard sprayer - 509 g a.s./hl - application paramaters from the actual orchard monitoring study

4 German model - air assisted orchard sprayer - 3.04 kg a.s./ha - with and without PPE

1.UK model for the determination of the operator exposure: tractor drawn air assisted orchard sprayer

PRODUCT DATA			
Product	Ziram		
Active ingredient	WG 76		
Concentration of the product(mg/ml or g/l)	760		
Formulation type	WG		
Concentration of a.s. in the spray (mg/ml)	3.04		
EXPOSURE DURING MIXING AND LOADING			
Container size (kg)	5		
Hand contamination/operation (ml or mg)	0.1		
Application dose (l formulation/ha)	4		
Work rate (ha/day)	30		
Number of operations per day	24		
Hand contamination (ml/day)	2.4		
Protective clothing	none		
Penetration of clothing (%)	100		
Dermal exposure to formulation(ml/day)	2.4		
EXPOSURE DURING SPRAY APPLICATION			
Application technique	Tractor mounted-air assisted		
Application volume(l spray/ha)	1000		
Volume of surface contamination(ml/hour)	400		
Distribution (%)	hands-10	trunk-65	legs-25
Clothing			
Penetration (%)	100	2	5
Dermal exposure(ml/hour)	10	5.2	5
Duration of exposure(hours)	6		
total dermal exposure to spray (ml/day)	121.2		

ABSORBED DOSE DUE TO THE MIXING/LOADING AND THE SPRAY APPLICATION		
	Mixing/loading	Application
Dermal exposure (ml/day)	2.4	121.2
Concentration of a.s.(mg/ml)	760	3.04
Dermal exposure to a.s.(mg/day).	1824	368.45
Percent absorbed(%)	5	30
Absorbed dose(mg/day)	91.2	110.53

INHALATION EXPOSURE DURING SPRAYING			
Inhalation exposure(ml/hour)	0.05		
Duration of exposure(hour)	6		
Concentration of a.s.(mg/ml)	3.04		
Inhalation exposure to a.s.(mg/day)	0.912		
Percent absorbed(%)	100		
Absorbed dose(mg/day)	0.912		
PREDICTED EXPOSURE	total	dermal	inhalation
absorbed dose(mg/day)	202.64	201.73	0.912
Operator body weight(kg)	60	60	60
operator exposure (mg/kg bw/day)	3.377	3.36	0.015
Total systemic exposure for a person(mg/kg bw/d)	3.377		
% AOEL	21109		

2.UK model for the determination of the operator exposure: tractor drawn air assisted orchard sprayer

PRODUCT DATA			
Product	Ziram		
Active ingredient	WG 76		
Concentration of the product(mg/ml or g/l)	760		
Formulation type	WG		
Concentration of a.s. in the spray (mg/ml)	3.04		
EXPOSURE DURING MIXING AND LOADING			
Container size (kg)	5		
Hand contamination/operation (ml or mg)	0.1		
Application dose (l formulation/ha)	4		
Work rate (ha/day)	30		
Number of operations per day	24		
Hand contamination (ml/day)	2.4		
Protective clothing	gloves		
Penetration of clothing (%)	1		
Dermal exposure to formulation(ml/day)	0.024		
EXPOSURE DURING SPRAY APPLICATION			
Application technique	Tractor mounted-air assisted		
Application volume(l spray/ha)	1000		
Volume of surface contamination(ml/hour)	400		
Distribution (%)	hands-10	trunk-65	legs-25
Clothing	gloves	permeable	permeable
Penetration (%)	10	2	5
Dermal exposure(ml/hour)	4	5.2	5
Duration of exposure(hours)	6		
total dermal exposure to spray (ml/day)	85.2		

ABSORBED DOSE DUE TO THE MIXING/LOADING AND THE SPRAY APPLICATION		
	Mixing/loading	Application
Dermal exposure (ml/day)	0.024	85.2
Concentration of a.s.(mg/ml)	760	3.04
Dermal exposure to a.s.(mg/day).	18.24	259
Percent absorbed(%)	5	30
Absorbed dose(mg/day)	0.912	77.7

INHALATION EXPOSURE DURING SPRAYING	
Inhalation exposure(ml/hour)	0.05
Duration of exposure(hour)	6
Concentration of a.s.(mg/ml)	3.04
Inhalation exposure to a.s.(mg/day)	0.912
Percent absorbed(%)	100
Absorbed dose(mg/day)	0.912
PREDICTED EXPOSURE	total
absorbed dose(mg/day)	79.52
Operator body weight(kg)	60
operator exposure (mg/kg bw/day)	1.325
Total systemic exposure for a person(mg/kg bw/d)	8.203
% AOEL	8250%

3. UK model for the determination of the operator exposure: parameters from the actual orchard monitoring study - USA conditions.

PRODUCT DATA			
Product	Ziram		
Active ingredient	WG 76		
Concentration of the product (mg/ml or g/l)	760		
Formulation type	WG		
Concentration of a.s. in the spray (mg/ml)	5.09		
EXPOSURE DURING MIXING AND LOADING			
Container size (kg)	5		
Hand contamination/operation (ml or mg)	0.1		
Application dose (l formulation/ha)	13.4		
Work rate (ha/day)	30		
Number of operations per day	80.4		
Hand contamination (ml/day)	8.04		
Protective clothing	no		
Penetration of clothing (%)	100		
Dermal exposure to formulation(ml/day)	8.04		
EXPOSURE DURING SPRAY APPLICATION			
Application technique	Tractor mounted-air assisted		
Application volume(l spray/ha)	2000		
Volume of surface contamination(ml/hour)	400		
Distribution (%)	hands-10	trunk-65	legs-25
Clothing			
Penetration (%)	100	2	5
Dermal exposure(ml/hour)	10	5.2	5
Duration of exposure(hours)	6		
total dermal exposure to spray (ml/day)	121.2		

ABSORBED DOSE DUE TO THE MIXING/LOADING AND THE SPRAY APPLICATION		
	Mixing/loading	Application
Dermal exposure (ml/day)	8.04	121.2
Concentration of a.s. (mg/ml)	760	5.09
Dermal exposure to a.s. (mg/day).	6110	617.15
Percent absorbed (%)	5	30
Absorbed dose (mg/day)	305.5	185.14

INHALATION EXPOSURE DURING SPRAYING			
Inhalation exposure (ml/hour)	0.05		
Duration of exposure (hour)	6		
Concentration of a.s. (mg/ml)	5.09		
Inhalation exposure to a.s. (mg/day)	1.52		
Percent absorbed (%)	100		
Absorbed dose(mg/day)	1.53		
PREDICTED EXPOSURE	total	dermal	inhalation
absorbed dose (mg/day)	492.2	490.66	1.53
Operator body weight (kg)	60	60	60
operator exposure (mg/kg bw/day)	8.20	8.17	0.025
Total systemic exposure for a person (mg/kg bw/d)	8.203		
% AOEL	51270		

4. German Model for the determination of the operator exposure

PRODUCT DATA			
Product	**Ziram 76 WG**		
Active ingredient	ziram		
Concentration of the product (g/l)	760		
Formulation type	WG		
Application technique			
Use rate (kg a.s./ha)	3.04		
Area treated per day (ha)	8		
Absorption factor (%)	100		
AOEL calculation	**AOEL oral**	**AOEL skin**	**AOEL inhalation**
NOAEL (mammal)(mg/kg/d)	3.2	100	3.2
Body weight (kg)	70	70	70
Assessment factor	200	100	200
	Otol	Dtol	Itol
Tolerable exposure(human)(mg/person/d)	1.12	70	1.12
SPECIFIC VALUES OF EXPOSURE FOR THE TYPE OF APPLICATION			
D*M(H) (mg/person*kg a.s.)	2	x 0.01(gloves)	
D*A(H) (mg/person*kg a.s.)	0.7	x 0.01 (gloves)	
D*A(B) (mg/person*kg a.s.)	9.6	x 0.05	
D*A(C) (mg/person*kg a.s.)	1.2		
I*M (mg/person*kg a.s.)	0.008		
I*A (mg/person*kg a.s.)	0.018		
ESTIMATION OF OPERATOR EXPOSURE			
Mixing			
DM(H)	48.64	0.486(gloves)	
IM	0.194	0.194	
Application			
DA(H)	17.02	0.170(gloves)	
DA(B)	233.5	11.67(0.05 : standard protective garment and sturdy footwear)	
DA(C)	29.18	29.18	
IA	0.438	0.438	
RISK ASSESSMENT			
DM(H)/Dtol	0.694	0.0069	
DA(H)/Dtol	0.243	0.002	
DA(B)/Dtol	3.335	0.1667	
DA(C)/Dtol	0.416	0.416	
IM/Itol	0.173	0.173	
IA/Itol	0.390	0.390	
Degree of exposure(E)	5.22	1.15	