ANNEX B

Ziram

B.8 Ecotoxicology

B.8.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.8.1.1 Acute oral toxicity (Annex IIA 8.1.1)

The Oral Toxicity (LC_{50}) of Ziram Technical to the Bobwhite quail (Hakin B. et al., 1990)
Guidelines :
U.S. Environmental Protection Agency (EPA) Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 71 -Avian and Mammalian testing, Section 71-1 Avian single-dose oral LD_{50} test, pages 33 to 37, dated October 1982.
GLP :
yes
Material and Methods :
Test Substance: **Ziram technical grade - Batch n° 8331 AA (Purity 98.5-99 %) in Corn oil**
Test species : **Bobwhite quail (*Colinus virginianus*)**
Sex, weight, age : 5 _ + 5_ birds/concentration
Applied concentrations : untreated control, **120, 204, 346, 588, & 1000** mg a.s. /kg body weight; because mortality in the first study was unexpectedly high, following concentrations were added to experimental design : 0, 24, 42, 71, 120 mg a.s./kg bw
Type of application : acute oral toxicity test
Time of exposure : one single application, monitoring during 14 days
Findings :
Table B.8.1.1-1 : Effects of ziram on bobwhite quails, after single dose exposition

Endpoints	**Doses (mg/kg bw)**								
	0	**24**	**42**	**71**	**120**	**204**	**346**	**588**	**1000**
Mortality (14 days after the application)	0 %	0 %	0 %	40 %	70 %	80 %	100 %	100 %	100 %
Clinical symptoms		watery excreta but otherwise remain ing in good health	watery excreta for up to 3 days following dosing						
			reversible clinical symptoms including ruffled feathers, huddling together, subdued behaviour, unsteadiness of gait, weakness and inability to stand; Signs increasing in intensity with dose level						
Body weight				_ : î	_ : î _ : î	_ : î	-	-	-
Food consumption					_ : î	_ : î _ : î	_ : î _ : î	_ : î	-
Post-mortem observations					Several birds with yellow mid-intestine				
				Gelatinous substance coating upper ventral area and liver; lower abdomen fluid filled	bruised right tigh, bird very thin	cream-like deposits on viscera and lungs	-	-	cream-like deposits on viscera and lungs

ìì :increase equivalent to control, no effect recorded; ì : reduced increase; î decrease

Endpoints :
LD_{50} = 97 mg a.s. /kg bw (95% Conf. limit, 74 - 121 mg/kg)
NOEL = 24 mg a.s./kg bw
Conclusions :
Study is acceptable.

B.8.1.2 Avian dietary toxicity (5day) (Annex IIA 8.1.2)

The Dietary Toxicity (LC_{50}) of Ziram Technical to the Mallard Duck (Hakin B. et al., 1992)
Guidelines :
U.S. Environmental Protection Agency (EPA) Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 71 -Avian and mammalian testing, Section 71-2 Avian dietary LC_{50} test, pages 37 to 43, dated October 1982.
GLP :
Yes
Material and Methods :
Test Substance: **Ziram technical grade - Batch n° 8331 AA (Purity: 98.5-99 %)**
Test species : **Mallard Duck (*Anas platyrhynchos*)**
Sex, weight, age : **10 birds/concentration x (4 concentrations plus 3 controls**); sex not determined, birds weight 76-77 g, 9-day old birds at start of study
Applied and measured concentrations : untreated control, **650, 1300, 2600, & 5200** mg/kg feed;
85 - 93% of nominal concentrations found in spiked samples.
Type of application : mixed in the diet
Time of exposure : Short-term feeding test (5 days with exposition by the feed + 7 days observations)
Findings :
Table B.8.1.1-1 : Effects of ziram on mallard ducks in a short term feeding test (5days)

Endpoints	**Doses (mg/kg feed)**				
	0	**650**	**1300**	**2600**	**5200**
Mortality (at the end of the study)	0 %	0 %	0 %	10 %	50 %
Clinical symptoms				subdued (days 5-6)	subdued and weak (days 4 to 10)
Body weight (days 0-5)	ìì	ì	ì	î	î
(days 5-8)	ìì	î	î	î	î
(days 8-12)	ìì	ìì	ìì	ìì	ìì
Feed consumption		reduction in all treated groups, very marked at highest dose, **palatability problem**			
Post-mortem observations					intestine dark in color

ìì :increase equivalent to control, no effect recorded; ì : reduced increase; î decrease
Endpoints :
LC_{50} (5d) 5156 mg a.s./kg feed (inf. conf. limit : 3740 mg a.s./kg feed)
NOEC (5d) = 1300 mg a.s./kg feed
Conclusions :

Study is acceptable. Ziram is known as repellent for the birds; It is possible that mortalities and body weight decreases resulted from starvation of birds and not from the direct toxicity of the a.s.

The Dietary Toxicity (LC_{50}) of Ziram Technical to the Bobwhite quail (Hakin B. et al., 1991)

Guidelines :

U.S. Environmental Protection Agency (EPA) Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 71 -Avian and mammalian testing, Section 71-2 Avian dietary LC_{50} test, pages 37 to 43, dated October 1982.

GLP :

Yes

Material and Methods :

Test Substance: **Ziram technical grade - Batch n° 8331 AA (Purity: 98.5-99 %)**

Test species : Bobwhite quail (*Colinus virginianus*)

Sex, weight, age : **10 birds/concentration x (4 concentrations plus 3 controls**); sex not determined, birds weight 11.6-12.2 g, 11-day old birds at start of study

Applied and measured concentrations : untreated control, **650, 1300, 2600, & 5200** mg/kg feed; of nominal concentrations found in spiked samples.

Type of application : mixed in the diet

Time of exposure : Short-term feeding test (5 days with exposition by the feed + 7 days observations)

Findings :

Table B.8.1.1-2 : Effects of ziram on bobwhite quails in a short term feeding test (5day)

Endpoints	**Doses (mg/kg feed)**				
	0	**650**	**1300**	**2600**	**5200**
Mortality (at the end of the study)	0 %	0 %	0 %	10 %	30 %
Clinical symptoms	-	-	-	- 1 bird subdued at day 1, recovered on day 2 - all birds subdued on days 5-6	subdued (day 3 to 6)
Body weight (days 0-5)	ìì	ìì	ìì	ì	î
(days 5-12)	ìì	ìì	ìì	ìì	ìì
Feed consumption (days 0-5)	=	=	=	î	î
(days 5-12)	=	=	=	=	=
Post-mortem observations	-	-	-	-	-

ìì :increase equivalent to control, no effect recorded; ì : reduced increase; î decrease

Endpoints :

LC_{50} (5d) > 5200 mg a.s./kg feed

NOEC (5d) = 1300 mg a.s./kg feed

Conclusions :

The clinical symptoms and the mortality in the 2600 and 5200 dose groups may be induced by the decrease of food consumption.

Study is acceptable.

B.8.1.3 Subchronic and reproductive toxicity (Annex IIA 8.1.3)

Not submitted. The study is required.

B.8.1.4 Acute oral toxicity of the preparations (Annex IIIA 10.1.1)

Not submitted.

B.8.1.5 Supervised cage or field trials (Annex IIIA 10.1.2)

Not submitted.

B.8.1.6 Acceptance of bait, granules or treated seeds by birds (palatability test) (Annex IIIA 10.1.3)

It is not clear in the dossier whether the notifiers want to support the seed treatment use. In this case, information on acceptance of treated seeds should be provided.

B.8.1.7 Effects of secondary poisoning (Annex IIIA 10.1.4)

Not submitted.

B.8.1.8 Summary of effects to birds - exposure and hazard assessment for birds (Annex IIIA 10.1)

Table B.8.1.8-1 : Summary of effects of ziram to birds.

Test species	**Test System**	**Duration of exposure**	**Results**	**References**
Colinus virginianus	acute oral toxicity	single application	LD_{50} = 97 mg/kg bw	Hakin B. Et al., 1990
Anas platyrhynchos	dietary toxicity	5 days	LC_{50} = 5200 mg/kg food	Hakin B. Et al., 1992
Colinus virginianus	dietary toxicity	5 days	LC_{50} > 5200 mg/kg food	Hakin B. Et al., 1991

TER acute and short term :
The risk assessment for birds is based on the following assumptions :
- Application in orchard of 3.04 kg a.s./ha (plums, peaches, cherries)
- Estimated initial residue in bird feed calculated in accordance with the method of Hoerger and Kenaga (1972)
- small birds (< 100 g bw) and large birds (> 100 g bw) scenarios were taken into account.

TER acute of 3 and 10 were calculated for small and large birds respectively. (Threshold value is 10)
TER short term of 55 were calculated for small and large birds (Threshold value is 10)

TER long term :
TER long term were not calculated since no reproduction study was available. The evaluation of this risk is necessary :
- Indeed the effects on the reproduction at concentrations lower than the concentrations causing unpalatatability of the food are not known.
- Continuous or repeated exposure is possible (4 to 6 applications made at 14 days interval in orchard)

It is therefore necessary to evaluate the effects of ziram on the reproduction of birds and the long term risk to birds living in orchard.

Seed treatment : No evaluation of the risks in the dossier.

Table B.8.1.8-2 : Estimated initial concentration of Ziram in potential feed of birds

Target crop	**Application rate (g a.s./ha)**	**Estimated initial residues (mg a.s./ kg food)**		
		Small seeds and small insects	**big insects and big insects**	**Leaves and leafy crops**
orchard	3.040	88.16	8.208	94.24

Tab. B.8.1.8-3 : Estimated oral uptake of Ziram by birds

Target crop	**Bird type**	**food consumed**	**food consumption (% bw)**	**Max. daily intake (mg a.s./kg bw / day)**
Orchard	small bird (<100 g)	small seeds and small insects	30	26.45
		big seeds and big insects	30	2.46
		leaves and leafy crops	30	28.27
Orchard	large bird (>100 g)	small seeds and small insects	10	8.81
		big seeds and big insects	10	0.82
		leaves and leafy crops	10	9.42

Tab. B.8.1.8-4 : Toxicity exposure ratios (TER's) for birds exposed to Ziram - worst cases

Bird type	**food consumed**	**Effect concentration**	**Estimated Theorical Exposure**	**Type of TER**	**Toxicity Exposure Ratio**
small bird	leaves and leafy crops	97 mg a.s./kg bw	28.27 mg a.s./kg bw	LD_{50}/ETE	3
		5200 mg a.s./kg feed	94.24 mg a.s./kg feed	LC_{50}/ETE	55
		- mg a.s./kg feed	- mg a.s./kg feed	NOEC/ETE	-

large bird	leaves and leafy crops	97 mg a.s./kg bw	9.42 mg a.s./kg bw	LD_{50}/ETE	10
		5200 mg a.s./kg feed	94.24 mg a.s./kg feed	LC_{50}/ETE	55
		- mg a.s./kg feed	- mg a.s./kg feed	NOEC/ETE	-

B.8.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)

B.8.2.1 Acute toxicity of the active substance and metabolites, degradation or reactions products to fish (Annex IIA 8.2.1)

The Acute Toxicity of Ziram Technical To Rainbow Trout (*Salmo gairdneri*) (Douglas M. et al., 1990)
Guidelines :
OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1
GLP :
Yes
Material and Methods :
Test substance : **Ziram technical grade - Batch n° 8331 AA (Purity: 98.9 %)**
Test species : **Rainbow Trout (*Salmo gairdneri*)**
Number of organisms, weight, length, : **10 fishes X 2 replicates per concentration** , 6.1 cm ± 0.7; 3.22 g ± 1.2,

Type of test : acute toxicity test (semi-static - 96 hours)
Applied and measured concentrations : **0, 0.17, 0.36, 0.75, 1.4, & 2.7 mg/liter**
76% of nominal concentrations found in spiked samples. (Mean recovery)
Test conditions :
temperature : 13°C,
pH : 7.6 to 7.9,
oxygen content : 10.3 mg O_2/l (0 h) to 10.1 mg O_2/l (96h)
Photoperiod : 16 hours light, 8 hours dark
Analytical methods : spectrophotometry
Findings :
Mortality : 25 % mortality at 1.4 mg/l; 100 % mortality at 2.7 mg/l
Behavioral observations : 50% fishes with increased pigmentation at 0.36 mg/l, in the other groups (0.75 to 2.7 mg/l) increased pigmentation, lethargy, loss of equilibrum and fish lying on bottom are observed.
Endpoints :
24 hour LC_{50} = 2.4 mg a.s./l (95% conf. limits, 1.8 - 3.3)
48 hour LC_{50} = 1.9 mg a.s./l (95% conf. limits, 1.7 - 2.1)
72 hour LC_{50} = 1.9 mg a.s./l (95% conf. limits, 1.7 - 2.1)
96 hour LC_{50} = 1.7 mg a.s./l (95% conf. limits 1.5 - 2.0)
NOEC = 0.17 mg a.s./l
Conclusion :
Ziram is toxic to rainbow trouts

The Acute Toxicity of Ziram Technical To Bluegill Sunfish (*Lepomis macrochirus*) (Douglas M. et al., 1990)
Guidelines :
OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1
GLP :
Yes
Material and Methods :
Test substance : **Ziram technical grade - Batch n° 8331 AA (Purity: 98.9 %)**
Test species : **Bluegill sunfish (*Lepomis macrochirus*)**
Number of organisms, weight, length : 10 fishes X 2 replicates per concentration; 1.01 g ± 0.4; 3.3 cm ± 0.6
Type of test : acute toxicity test (continuous renewal - 96 hours)
Applied and measured concentrations : **0, 0.0046, 0.0076, 0.015, 0.022, & 0.025 mg a.s./liter**
86 - 108 % of nominal concentrations found in spiked samples.
Test conditions :
temperature : 21 °C,
pH : 7.5 to 7.7,
oxygen content : 8.8 mg O_2/l (0 h) to 8.6 mg O_2/l (96h)
Photoperiod : 16 hours light, 8 hours dark
Analytical methods : GC with head-space after CS_2 transformation of a.s.
Findings :
Mortality : 5 % mortality at 0.0076 mg/l; 60 % mortality at 0.015 mg/l; 90 % mortality at 0.022 and 0.025 mg/l

Behavioral observations : 2 fishes lethargic or lying on bottom at dose 0.0076 mg/l. In the other groups (0.015 to 0.025 mg/l) following symptoms were observed at 24-96 hours : lethargy, lying on bottom, swimming at surface, moribund
Endpoints :
24 hour LC_{50} > 0.022 mg a.s./l
48 hour LC_{50} = 0.016 mg a.s. /l (95% conf. limits, 0.011 - 0.023)
72 hour LC_{50} = 0.12 mg a.s./l (95% conf. limits, 0.0099 - 0.014)
96 hour LC_{50} = 0.0097 mg a.s./l (95% conf. limits, 0.0086 - 0.011)
NOEC = 0.0046 mg a.s./l
Conclusion :
Ziram is very toxic to bluegill sunfish

Examination for acute toxicity of ziram in carp at exposition of 96 hours in the bath fluid (Dickhaus S., 1980)
This study was realized in a static system. No information about the origin and the purity of the a.s. is given. No information about the concentrations of a.s. in water at the end of the test
LC_{50} (96 hours - carp) = 2.28 mg/l

B.8.2.2 Fish early life stage toxicity (Annex IIA 8.2.2.1)

Not submitted.

Chronic toxicity test study with fish is required because
- repeated exposure of the aquatic environment is possible : main uses are in orchard, with several applications during the growing season.
- the acute toxicity is very high. We can therefore expect that chronic effects would occur at very low concentrations.
- the information on the degradation behaviour and on the degradation rate in water is very scarce. At this stage accurate short term PEC cannot be calculated.

B.8.2.3 Fish juvenile growth test (Annex IIA 8.2.2.2)

Not submitted. See point B.8.2.2

B.8.2.4 Fish life cycle test (Annex IIA 8.2.2.3)

Not submitted. See point B.8.2.2

B.8.2.5 Bioaccumulation potential in fish (Annex IIA 8.2.3)

Not submitted. The realization of this study is required when log Pow is higher than 3.
In absence of a valid log Pow determination study, the requirement of a bioaccumulation study cannot be waived.

B.8.2.6 Acute toxicity to invertebrates (Annex IIA 8.2.4)

The Acute Toxicity of Ziram Technical To Daphnia magna (Douglas M. et al. 1990)
Guidelines :
OECD Guideline No. 202 Part 1 and EPA Pesticide Guidelines Subdivision E, Section 72-2.
GLP :
Yes
Material and Methods :
Test substance : **Ziram technical grade - Batch n° 8331 AA (Purity 98.9 %)**
Test species : Waterflea (*Daphnia magna*)

Number of organisms, age : 10 animals X 2 replicates per concentration; animals younger than 24 hours
Type of test : acute toxicity test (48 hours)
Applied and measured concentrations : 0, 0.0012, 0.0012, 0.0026, 0.0017, 0.0072, 0.0055, 0.0094, 0.015, 0.032, 0.048, 0.096 mg/l
105 - 107 % of nominal concentrations found in spiked samples.
Test conditions :
temperature : 21 °C,
pH : 8.0,
oxygen content : 8.9 mg O_2/l (0 h) to 8.6 mg O_2/l (48h)
Photoperiod : 16 hours light, 8 hours dark
Analytical methods : GC with head-space after CS_2 transformation of a.s.
Findings :
Endpoints :
24 hour EC_{50} = 0.15 mg a.s./l (95% conf. limits 0.065 -0.34 mg/l)
48 hour EC_{50} = 0.048 mg a.s./l (95% conf limits 0.034 - 0.068 mg/l)
24 hour NOEC = 0.015 mg a.s./l
48 hour NOEC = 0.0017 mg a.s./l
Lowest concentration resulting in 100% immobility = 0.096 mg a.s./l
Conclusion :
Ziram is very toxic to *Daphnia magna*

B.8.2.7 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

Not submitted.

Chronic toxicity test study with aquatic invertebrate is required because
- repeated exposure of the aquatic environment is possible : main uses are in orchard, with several applications during the growing season.
- the acute toxicity is very high. We can therefore expect that chronic effects would occur at very low concentrations.
- the information on the degradation behaviour and on the degradation rate in water is very scarce. At this stage accurate short term PEC cannot be calculated.

B.8.2.8 Effects on algal growth (Annex IIA 8.2.6)

Ziram - Toxicity to the freshwater green alga, *Selenastrum capricornutum* (Hoberg J., 1995)
Guidelines :
US EPA FIFRA guidelines 122-2 and 122-3
GLP :
Yes
Material and Methods :
Test substance : **Ziram technical grade - Batch n° 4713 AA (Purity 98 %)**
Test species : green alga *Selenastrum capricornutum* strain 1648
Number of organisms, replicates : 0.3 X 10^4 cells/mL at initiation. 3 replicates/concentration
Type of test : 96-hour static system
Applied and measured concentrations : 0, 0.039, 0.075, 0.15, 0.30, 0.60 mg a.s./l
98-107% of nominal concentrations found in spiked samples at begin of the test
63-86 % of nominal concentrations found in spiked samples at the termination of the test
Test conditions :
Temperature and light intensity were monitored. PH and conductivity of water were measured prior to test inititiation and a the terminination of th 5-day exposure period.
temperature : 24-25 °C,
pH : 7.5-8.8
Continuous illumination at 3800 to 5100 lux

shaking at 100 rpm
Analytical methods :
HPLC
Findings :
Observations :
Control and solvent control averaged 270 and 331 104 cells/mL at test termination.
Cell fragments were observed at the concentrations 0.075 to 0.6 mg a.s./l
Bloated cells were observed at the concentrations 0.15 and 0.30 mg a.s./l
Cells exposed to 0.039 mg a.s./l were observed to be normal throughout the exposure.
Endpoints :
EC_{25} (72 h) = 0.062 mg a.s./l (95%CL = 0.025-0.14)
EC_{50} (72 h) = 0.084 mg a.s./l (95%CL = 0.034-0.19)

EC_{05} (96 h) = 0.033 mg a.s./l (95%CL = 0.014-0.068)
EC_{25} (96 h) = 0.045 mg a.s./l (95%CL = 0.020-0.095)
EC_{50} (96 h) = 0.066 mg a.s./l (95%CL = 0.030-0.14)
Conclusions:
Ziram is very toxic to *Selenastrum capricornutum*

B.8.2.9 Effects on the organisms of the sediments (Annex IIA 8.2.7)

Not submitted. The study is required.

B.8.2.10 Effects on aquatic plants (Annex IIA 8.2.8)

The study is not required because ziram is not an herbicide.

B.8.2.11 Acute toxicity of the preparations (Annex IIIA 10.2.1)

Not submitted.

B.8.2.12 Microcosm and mesocosm study (Annex IIIA 10.2.2)

Not submitted. The study is required because
- the TERacute are lower than the trigger value of 100 (0.048 to 1.5 depending on organism and drift value).
- No information on chronic toxicity is provided.

B.8.2.13 Residue data in fish (Annex IIIA 10.2.3)

Not submitted.

B.8.2.14 Supplementary studies of toxicity to fish and aquatic invertebrates (Annex IIIA 10.2.4)

Not submitted.

B.8.2.15 Summary of effects to water organisms

The acute toxicity tests provided showed that ziram is very to toxic to all groups of aquatic organisms (fish, aquatic invertebrate, alga).

Chronic toxicity tests with fish, aquatic invertebrate and alga are necessary :
- The evaluation of the long term risk is impossible with the data already available.
- The EC_{50} for the three groups of organisms are < 0.1 mg a.s./l
- The risk due to repeated application can not be excluded.

The notifiers must also address the following points : the bioaccumulation, the toxicity to sediment dwelling arthropods, the micro-mesocosm and the TER acute and long term.

Table B.8.2.15-1 : Summary of effects of ziram to water organisms

Test species	Test system	Duration of exposure	Results	References
Salmo gairdneri	Semi-static	96 hours	LC_{50} (96h) = 1.7 mg a.s./l NOEC = 0.17 mg a.s./l	Douglas M. et al., 1990
Lepomis macrochirus	Continuous renewal	96 hours	LC_{50} (96h) = 0.0097 mg a.s./l NOEC = 0.0046 mg a.s./l	Douglas M. et al., 1990
Daphnia magna	Static - immobilisation test	48 hours	EC_{50} (48h) = 0.048 mg a.s./l NOEC = 0.0017 mg a.s./l	Douglas M. et al., 1990
Selenastrum capricornutum	static	96 hours	EC_{50} (96 h) = 0.066 mg a.s./l	Hoberg J., 1995

B.8.2.16 Risk assessment for aquatic organisms (Annex IIIA 10.2)

The following assumptions were made to assess the risks for water organisms :
- application of 3.040 kg a.s./ha in orchard
- drift calculated according to Ganzelmeier (BBA)
- water depth = 30 cm

TER acute in the worst case situation (orchard - early growth stage - 5 or 20 m drift) are far below the threshold value of 100 for fish and aquatic invertebrates, of 10 for alga.

The evaluation of a long term risk (taking into account repeated applications) is impossible with the data available at this time.

Table B.8.2.16-1 : Estimated initial concentrations of ziram in surface water - Drift scenarios in orchard

Target crop	**Application rate (g a.s./ha)**	**Distance (m)**	**Drift (%)**	**Estimated initial concentration (mg a.s./l water)**
Orchard (early growth stage)	3040	0	100	1.013 (*)
		5	20	0.203
		20	4	0.040
Orchard (late growth stage)	3040	0	100	1.013 (*)
		5	10	0.101
		20	1.5	0.015

(*) : unrealistic situation ; only to help the reader

Table B.8.2.13-3 : Theoretical Exposure Ratio for aquatic organisms exposed to ziram -Worst case scenario

Target crop	**Distance (m)**	**Test species**	**Effect concentration**	**Estimated initial concentration (mg a.s./l)**	**TER**
Orchard (early growth stage)	5	*Lepomis macrochirus*	0.0097 mg a.s./l	0.203	0.048
	20		0.0097 mg a.s./l	0.040	0.24
Orchard (early growth stage)	5	*Daphnia magna*	0.048 mg a.s./l	0.203	0.24
	20		0.048 mg a.s./l	0.040	1.2
Orchard (early growth stage)	5	*Selenastrum capricornutum*	0.066 mg a.s./l	0.203	0.32
	20		0.066 mg a.s./l	0.040	1.5

B.8.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3.1)

Table B.8.3-1 : Toxicity figures taken into account in the mammals assessment

Test species	Test System	Duration of exposure	Results	References
rat	acute oral toxicity	single application	LD_{50} = 267 mg/kg bw	Liggett et al., 1989a
rat	dietary toxicity	28 day and 90 day studies (studies taken into account for the determination of the ADI)	NOAEL = 3 mg/kg food	Dickhaus et al. 1980 Powell et al., 1994

TER acute and short term :
The risk assessment for small mammals is based on the following assumptions :
- Application in orchard of 3.04 kg a.s./ha (plums, peaches, cherries)
- Estimated initial residue in bird feed calculated in accordance with the method of Hoerger and Kenaga (1972)
- small mammals (< 100 g bw)

TER acute of 9.4 was calculated for small mammals. (Threshold value is 10)
TER short term of 0.03 was calculated for small mammals (Threshold value is 10)

Seed treatment : No evaluation of the risks in the dossier.

Table B.8.3-2 : Estimated initial concentration of Ziram in potential feed of small mammals

Target crop	Application rate (g a.s./ha)	Estimated initial residues (mg a.s./ kg food)		
		Small seeds and small insects	big insects and big insects	Leaves and leafy crops
orchard	3.040	88.16	8.208	94.24

Table B.8.3-3 : Estimated oral uptake of Ziram by small mammals

Target crop	Animals	food consumed	food consumption (% bw)	Max. daily intake (mg a.s./kg bw / day)
Orchard	small mammal (<100 g)	small seeds and small insects	30	26.45
		big seeds and big insects	30	2.46
		leaves and leafy crops	30	28.27

Tab. B.8.3-4 : Toxicity exposure ratios (TER's) for small mammals exposed to Ziram - worst cases

Animal	**food consumed**	**Effect concentration**	**Estimated Theorical Exposure**	**Type of TER**	**Toxicity Exposure Ratio**
small mammal	leaves and leafy crops	267 mg a.s./kg bw	28.27 mg a.s./kg bw	LD_{50}/ETE	9.4
		3 mg a.s./kg feed	94.24 mg a.s./kg feed	NOAEL/ETE	0.03

B.8.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.3.2)

B.8.4.1 Acute toxicity to bees (Annex IIA 8.3.1.1)

The acute contact toxicity to honey bees of technical ziram (J. Cole, 1989)
The acute contact and oral toxicity to honey bees of technical ziram (Cole J., 1990)
Guidelines :
U.S. Environmental Protection Agency (EPA) Pesticide Assessment Guidelines, Subdivision L, Series 141-1.
GLP :
Yes
Material and Methods :
Test substance : **Ziram technical grade - Batch n° 8331 AA (Purity: 98.5 %)**
Test species : Honeybees (*Apis mellifera L.*); worker bees
Number of organisms : 10 groups of 10 bees at maximum concentration 100 µg/bee; 2 groups of 10 bees for control
Type of test : Acute contact toxicity and acute oral toxicity test (48 hours)
*Applied concentrations :*100 µg a.s. /bee (nominal) and solvent control (a range finding test made at the doses 0.01 µg/bee to 100 µg/bee and show no dose-related mortality even at the highest dose)
Exposure route :
- after anesthesis with CO_2 1 µl dilution of ziram is placed on the ventral surface of the thorax of each bee; Ziram was diluted in dimethylsulfoxide
- A single dose of 0.2 ml to each group of 10 bees was administered
Feeding : 20 % sucrose in water ad libitum
Test conditions :
Temperature : 24 ± 1 °C
Humidity was not recorded (40-80 %)
Findings :
Endpoints :
LD_{50} (48h) contact > 100 µg a.s. /bee (nominal)
NOEL (48h) contact > 100 µg a.s. /bee (nominal)

LD_{50} (48h) oral > 100 µg a.s. /bee (nominal)
NOEL (48h) oral > 100 µg a.s. /bee (nominal)
Conclusion :
Ziram is not toxic to bees up to 100 µg/ bee

B.8.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)

Not required since Ziram is not an insect growth-regulating substance

B.8.4.3 Acute toxicity of the preparations to bees (Annex IIIA 10.4.1)

Not required.

B.8.4.4 effects on bees of residues on crops (Annex IIIA 10.4.2)

Not required.

B.8.4.5 Cage tests (Annex IIIA 10.4.3)

Not required.

B.8.4.6 Field tests to investigate special effects (Annex IIIA 10.4.4)

Not required.

B.8.4.7 Tunnel testing to investigate effects of feeding on contaminated honey (Annex IIIA 10.4.5)

Not required.

B.8.4.8 Exposure and risk assessment for bees (Annex IIIA 10.4)

Table B.8.4.8-1 : Toxicity and risk assessment for bees

Test species	Test system	Results	Hazard Ratio	References
Honeybee *(Apis mellifera L)*	Acute contact toxicity test	LD_{50} > 100 µg a.s./ bee.	Qhc = 3040/100 = 30.4	**(J. Cole, 1990)**
	Acute oral toxicity test	LD_{50} > 100 µg a.s./ bee.	Qho = 3040/100 = 30.4	

The Qhc and Qho ratios were calculated taking into the highest application rate in orchard (3040 g a.s./ha) Both ratios are < 50 indicating that the risk to bees is negligible.

B.8.5 Effects on other arthropods species (Annex IIA 8.3.2; Annex IIIA 10.5)

B.8.5.1 Effects of the active substance on non-target terrestrial arthropods (Annex IIA 8.3.2)
B.8.5.2 Effects of the formulations on non-target terrestrial arthropods (laboratory, semi-field tests) Annex IIIA 10.5.1)

Not submitted. Since effects were observed in the field studies the RMS would not propose to require laboratory and semi-field studies which have as sole objective the confirmation of the high toxicity of the a.s.

B.8.5.3 Effects of the formulations on non-target terrestrial arthropods (field tests) Annex IIIA 10.5.2)

Toxicity of ziram to the predatory mite *Typhlodromus pyri* (Research Station of Gorsem, 1995)
Guideline:
IOBC/Working group “Pesticides and beneficial organisms”
GLP :
No
Material and Methods :
Test substance :
ZIRAM, WG containing 760 g ziram /kg
Test species :
The trials were realized in orchards with a sufficient number of *Typhlodromus pyri*, at least a mean value of 1 predatory mite on 2 leaves is required. All the predatory mites in the testing orchards are from the same origin, an organophosphates and carbamates resistant strain from the Netherlands
Experimental design :
- 2 field trials in orchard were performed in 1993 and 1995; the trials were designed as randomized blocks with 4 replications of at least 10 trees., formulations containing captan, mancozeb,... were tested.
- In each object 4 times 50 leaves were examined with binocular. The Henderson-Tilton formula was applied on these results. Henderson-Tilton criteria < +25% means that the product is harmless
- Maximum and minimum temperatures, sunshine and rainfall were recorded .
Findings :

Table B.8.5.3-1 : Overview of 2 trials in orchard evaluating the effects of ZIRAM on *Typhlodromus pyri*

Location	Kozen (Belgium)	Rummen (Belgium)
Crop	apple Jonagold	apple Jonagold
Experimental design	4 replicates/treatment 10 trees/object	4 replicates/treatment 36 trees/object
Number of applications	4 applications on 20/07/93 29/07/93 04/08/93 11/08/93	7 applications between 13/06/95 and 14/08/95
Application rate	2280 g a.s/ha 1500 l water/ha	2280 g a.s./ha 1500 l water/ha
Henderson-Tilton coefficient	E(after 2 appl.) = **37.0%** E(after 4 appl.) = **45.8 %**	E(after 2 appl.) = -58.9 % E(after 5 appl.) = **81.9 %** E(21 d after last appl.) = **75.2%**

bold : E = 25-49 % slightly harmful; E= 50-74 % moderately harmful; E ³ 75% harmful

Conclusions :
According to the standards for field trials of the IOBC working group, the toxicity of ziram for *Typhlodromus pyri* in the field varies between harmless (< 25%) and harmful depending upon the number of treatments
The climatological conditions (temperature) and the history of the orchard (sensitivity to products) may explain the differences of effects between the years. A limitation of the number of applications (2 applications/year) seems therefore necessary.

Total fauna experiment for the evaluation of the side effects of UCB Ziram on beneficial arthropods (Research Station of Gorsem, 1996)
Guideline:
-
GLP :
No
Material and Methods :
Test substance :
ZIRAM, WG containing 760 g ziram /kg
Test species :
The effects of ziram were studied on the arthropod fauna living on *Alnus glutinosa* (beneficials are more abundant on this species than on apple or pear trees)
Experimental design :
the study was performed with 3 replicates (3 trees/object). 3 applications of ziram were made.
Directly after spraying plastic sails were placed under the trees. Fallen insects were sampled 1 hour, 1 day, 2 days, 4 days and 7 days after the treatments and were determined. Dichlorvos was used as toxic control
The effects of the a.s. were calculated with the Steiner formula.
Maximum and minimum temperatures, sunshine and rainfall were recorded .

Table B.8.5.3-1 : Overview of a field trial on *Alnus glutinosa* evaluating the effects of ZIRAM WG on a wide range of beneficial arthropods

Location	Tongeren (Belgium)
Crop	*Alnus glutinosa*
Experimental design	3 replicates/treatment 3 trees/object
Number of applications	3 applications on 12/07/93 15/07/93 19/07/93
Application rate	0.2% formulation sprayed until runoff corresponds to 2280 g a.s./ha in orchard

Findings :

Table : Main results of a field trial on *Alnus glutinosa* evaluating the effects of ZIRAM on a wide range of beneficial arthropods

Beneficial arthropods		**Steiner coefficient**
Anthocoridae	adults	E = -2%
	nymphs	**E = 25 %**
Dermaptera	adults	**E = 26 %**
Chrysopidae	adults	E = -182 %
	nymphs	**E = 41 %**
Coccinellidae	adults	**E = 28 %**
	nymphs	E = 9 %
Syrphidae	adults	**E = 26 %**
Hymenoptera (Ichneumonids and Chalconids)	adults	E = 19 %
Arachnida	adults and nymphs	**E = 35 %**

bold : E = 25-49 % slightly harmful

Conclusions :

This experiment showed that ziram is slightly harmful for several groups of beneficial arthropods after 3 applications in *Alnus glutinosa* mimicking an orchard situation with important arthropod populations.

B.8.5.4 Summary of effects, exposure and hazard assessment for non-target terrestrial arthropods

Two field tests were performed. They show that ziram is slightly harmful to harmful to a wide range of beneficial arthropods in situations close to actual use.

The studies were not realized at the highest recommended dose of 3.040 kg a.s./ha
No information on the soil dwelling arthropods was provided

Table B.8.5.4-1 : Summary of effects of ziram to non-target terrestrial arthropods (field tests)

Test species	Test system	Results			References
Typhlodromus pyri	apples 4 applications 2280 g a.s./ha	E(after 2 appl.) = **37.0%** E(after 4 appl.) = **45.8 %**			Research Station of Gorsem, 1995
	apples 7 applications 2280 g a.s./ha	E(after 2 appl.) = -58.9 % E(after 5 appl.) = **81.9 %** E(21 d after last appl.) = **75.2%**			
Total arthropod fauna	*Alnus glutinosa* 3 applications 2280 g a.s./ha	Anthocoridae	adults	E = -2%	Research Station of Gorsem, 1996
			nymphs	**E = 25 %**	
		Dermaptera	adults	**E = 26 %**	
		Chrysopidae	adults	E = -182 %	
			nymphs	**E = 41 %**	
		Coccinellidae	adults	**E = 28 %**	
			nymphs	E = 9 %	
		Syrphidae	adults	**E = 26 %**	
		Hymenoptera (Ichneumonids and Chalconids)	adults	E = 19 %	
		Arachnida	adults and nymphs	**E = 35 %**	

bold : E = 25-49 % slightly harmful; E= 50-74 % moderately harmful; E ³ 75% harmful

B.8.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.3.6)

B.8.6.1 Acute toxicity to earthworms (Annex IIA 8.4.1)

The Acute Toxicity of Ziram Technical To Earthworms (Handley J. et al., 1993)
Guidelines :
OECD Guideline No. 207 and EEC Commission Directive 87/302/EEC
GLP :
Yes
Material and Methods :
Test substance : **Ziram technical grade - Batch n° V528/8331 AA (Purity: 98.3 %**)
Test species : Earthworms (*Eisenia foetida*);
Number of organisms, weight, length, age : 10 worms X 4 replicates / concentration; 0.41 g ± 0.09
Type of test : acute toxicity test in artificial soil (14 days)
Applied concentrations : **0,100, 180, 320, 560, & 1000 mg/kg soil** mg a.s./kg (nominal);
positive control : chloroacetamide
Soil type and test conditions :
Test substrate : 69% industrial quartz sand, 20% kaolinite; 10% moss peat; 1% calcium carbonate
water content : 28-30 %
pH : 6.2 ± 0.1
Temperature : 21 °C
Light regime : continuous at 600 lux
Findings :
Mortality : 88% mortality at 180 mg/kg soil; 100% at the other concentrations (320 to 1000 mg/kg soil)
Observations : no behavioural reactions to exposure, no difference of weight between control and dose 100 mg/kg soil
Endpoints :
LC_{50} (7 d) 190 mg a.s./kg substrate (confid. limits : 170-210)
LC_{50} (14d) 140 mg a.s./kg substrate (confid. limits : 140-150)
LC0 (14d) = 100 mg a.s./kg substrate
NOEC (14d) = 100 mg a.s./kg substrate
Conclusion :
Study is acceptable.

B.8.6.2 Sublethal effects on earthworms (Annex IIA 8.4.2)

Not submitted. This study is required since repeated exposure of the earthworms can not be excluded.

B.8.6.3 Acute toxicity of the formulations to earthworms (Annex IIIA 10.6.1.1)

Not submitted. Not required.

B.8.6.4 Sublethal effects of the formulation on earthworms (Annex IIIA 10.6.1.2)

Not submitted. Not required.

B.8.6.5 Field tests - residue content of earthworms (Annex IIIA 10.6.1.3)

Not submitted. No required.

B.8.6.6 Summary and risk assessment for earthworms (Annex III, 10.6.1.1)

One acute toxicity study with *Eisenia foetida* was realized. A TER acute of 69 was calculated (trigger value is 10) showing that the risk to earthworm is low. The evaluation of the risks resulting from repeated applications should be performed.

Table B.8.6.6-1 : Summary of effects of ziram for earthworms

Test species	**Test system**	**Duration of exposure**	**Results (mg/kg soil)**	**References**
Earthworm *(Eisenia foetida)*	Acute toxicity	14 days	LC_{50} = 140 mg a.s./kg soil NOEC = 100 mg a.s./kg soil	Handley et al., 1993

The following assumptions were made to assess the risks for earthworms :
- application rate of 3.040 kg a.s./ha in orchard
- 50 % of the deposit reaching the soil surface
- dispersion in a 5 cm soil layer

Table B.8.6.6-2 : Risk assessment for the earthworms

Test species	**Effect concentration (mg a.s. kg soil)**	**Estimated initial concentration (mg a.s. /kg soil)**	**TER**
Earthworm *(Eisenia foetida)*	LC_{50} = 140 NOEC = 100	2.03	140/2.03 = 69

B.8.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

Not required. It is expected that the DT90 soil < 100 days.

B.8.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

B.8.8.1 Impact of the active substance on soil microbial activity (Annex IIA 8.5)
B.8.8.2 Impact of the formulations on soil microbial activity (laboratory) (Annex IIIA 10.7.1)
B.8.8.3 Further laboratory, glasshouse of field testing to investigate impact on soil microbial activity (Annex IIIA 10.7.2)
B.8.8.4 Summary of studies on non-target micro-organisms - exposure and hazard assessment for non-target micro-organisms

Not submitted. The effects on soil non-target micro-organisms must be evaluated
The risk assessment must be performed.

B.8.9 Effects on other non-target organisms (flora and fauna) believed to be at risk

Not required

B.8.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Not submitted. The point must be addressed by the notifiers.

B.8.11 References relied on

Ecotoxicology

Annex point(s) Dir 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 8.3.1	Cole J.	The acute contact toxicity to honey bees of technical Ziram. HRC - Report n° ZIR 21/891454 - 5 March 1990. Submitted by UCB-Elf Atochem Agri	yes	unpublished protected
IIA 8.3.1	Cole J.	The acute contact and oral toxicity to honey bees of technical Ziram. HRC - Report n° ZIR 21/891454 - September 1990. Submitted by UCB-Elf Atochem Agri	yes	unpublished -
IIA 8.2.4	Douglas M.T. *et al*	The acute Toxicity of Ziram technical to *Daphnia magna*. HRC - Report n° ZIR 20(a)/901625 - 3 Sept 1991c Submitted by UCB-Elf Atochem Agri	yes	unpublished -
IIA 8.2.1	Douglas M.T. *et al*	The acute toxicity of Ziram technical to Bluegill Sunfish (*Lepomis macrochirus*) HRC - Report n° ZIR 20(c)/901626 - 3.Sept 1991b Submitted by UCB-Elf Atochem Agri	yes	unpublished protected
IIA 8.2.1	Douglas M.T. *et al*	The acute toxicity of Ziram technical to Rainbow Trout (*Oncorhynchus mykiss*) HRC - Report n° ZIR20(b)/89117- 3 Sept 3, 1991a Submitted by UCB-Elf Atochem Agri	yes	unpublished protected
IIA 8.1.2	Hakin B. *et al*	The dietary toxicity of Ziram technical to the Bobwhite quail. HRC - Report n° ZIR 19/901457 - 13 Jan 1992a Submitted by UCB-Elf Atochem Agri	yes	unpublished -
IIA 8.1.2	Hakin B.*et al*	The dietary toxicity of Ziram technical to the Mallard duck . HRC - Report n° ZIR 18/901456 - 7 Jan 1992b Submitted by UCB-Elf Atochem Agri	yes	unpublished -
IIA 8.1.1	Hakin B. *et al*	Acute oral toxicity of Ziram technical to the bobwhite quail. HRC - Report n°. ZIR 17/90566 - 3 Oct 1990. Submitted by UCB-Elf Atochem Agri	yes	unpublished -
IIA 8.4	Handley J.W. et al	The acute toxicity of Ziram technical to earthworms. Safepharm Lab, - Report n° 378/20 - 18 March 1993 Submitted by UCB-Elf Atochem Agri	yes	unpublished -
IIA 8.2.6	Hoberg, J.	Ziram - Toxicity to the green alga, *Selenastrum capricornutum* Springborn lab. -	yes	unpublished

Annex point(s) Dir 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
		Report No : 95-7-5971 1995 Submitted by UCB-Elf Atochem Agri		protected
IIA 8.2.1	Not specified	Examination for acute toxicity of Ziram in carp at exposition of 96 hours in the bath fluid Pharmatox 140-80 - September 1980 Submitted by UCB-Elf Atochem Agri	no	unpublished protected
IIA 8.3.2	Not specified	Toxicity of Ziram to the predatory mite Typhlodromus pyri Research Station of Gorsem - 1995 Submitted by UCB-Elf Atochem Agri	no	unpublished protected
IIA 8.3.2	Not specified	Total fauna experiment for evaluation of side effects of Ziram on beneficial arthropods Research Station of Gorsem - 1996 Submitted by UCB-Elf Atochem Agri	no	unpublished protected