ANNEX B

Ziram

B.9 Ecotoxicology

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.3 Subchronic and reproductive toxicity (Annex IIA 8.1.3)

Ziram Technical : A reproduction study with the Northern bobwhite (Frey, et al., 2000)
Analytical method verification for the determination of ziram in avian diet (Martin et al., 2001)
Guidelines :
OECD guideline 206
GLP :
Yes
Material and Methods :
Test substance : ziram technical (reported purity of 97%)
Test species : Northern bobwhite *(Colinus virginianus)*
Sex, weight, age : 16 pens of 1 male + 1 female per treatment group, weight of 175-229 g at test initiation, 29 weeks of age at test initiation
Applied concentrations : avian diet samples were fortified with 0, 125, 250, 500 mg a.s./kg in the feed. The recoveries were respectively 82%, 87% and 95%.
Time of exposure : Approximately 20 weeks : pre-photostimulation : 7 wk, pre-egg laying with photostimulation : 2 wk, egg laying : 11 wk

Findings :
Table B.9.1.3-1 : Major effects of ziram observed during the reproduction study of *Colinus virginianus*

	Doses (mg/kg feed)			
	0	125	250	500
Adults				
Mortality	No treatment related mortality (1 dead bird at 125 mg/kg)			
Clinical signs	No gross pathological observations (no effects on testis and ovary)			
Body weight	No treatment related change			
Food consumption	No treatment related change			
Reproduction Parameters				
Number of replicates	16	15	16	16
Total eggs laid	712	636	732	720
Eggs cracked/eggs laid (%)	1	2	2	2
Shell thickness (mean ± SD)	0.228 ± 0.011	0.230 ± 0.013	0.233 ± 0.011	0.234 ± 0.015
Viable embryos/eggs set (%)	88	92	91	92
Live 3-week embryos/viable embryos (%)	100	98	100	97
Hatchlings/ Live 3-week embryos (%)	96	91	96	87*
14-day old survivors/hatchlings (%)	98	95	97	96
Hatchlings/ eggs set (%)	85	83	87	80
14-day old survivors/eggs set (%)	83	79	85	77
Eggs laid/hen	45	42	46	45
Eggs laid/hen/day	0.48	0.46	0.49	0.48
14-day old survivors/hen	34	29	34	31

Conclusions :
There were no treatment related effects on mortality, clinical signs, body weight, food consumption, and reproductive performances
The NOEC (*Colinus virginianus*, Reproduction, 20 week) =500 mg a.s./kg feed or 36.6 mg a.s./kg bw/d

B.9.1.8 Summary of effects to birds - exposure and risk assessment for birds (Annex IIIA 10.1)

The notifier recalculates the following TER (the application rate has been reduced from 3.04 to 2.28 kg a.s./ha; the new NOEL reproduction of 500 mg a.s./kg food has been considered.

Toxicity exposure ratios for birds				
Appl. rate (g a.s./ha)	Organism	Time scale	TER	Annex VI trigger
2280	Small insectivorous bird	acute	**4.9**	10
	Small insectivorous bird	short term	78	10
	Small insectivorous bird	long term	7.5	5

The notifier considers that the diet is composed not only of small insects or contaminated feed, the population of birds spent less than 61% of potential foraging time in orchard, that residues on crop would be more limited (Data of Hoerger and Kenaga would over-estimate the actual feed contamination).

The short term TER (78) indicates that the consumption of contaminated feed during a few days would not endanger birdlife. On this basis the rational presented by the notifier is considered as acceptable.

B.9.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.2 Chronic toxicity to fish (Annex IIA 8.2.2.1)

Sublethal effects of Ziram 76WG to bluegill sunfish (*Lepomis macrochirus*) after a fourfold application to a water-sediment system (Memmert, 2001)
Guidelines :
OECD N° 215, some modifications to mimic more realistic use conditions : 4 applications at 7 days interval in a water/sediment system
GLP :
Yes
Material and Methods :
Test substance : Ziram 76 WG, containing 75.58% ziram
Test species : bluegill sunfish (*Lepomis macrochirus*)
Number of organisms, weight, length, loading :
10 fishes per concentration. Initial loading rate was ± 0.1 g/l; at the end ± 0.15 g/l.
Type of test :

Water sediment systems: one tank with a water volume of 180 liters was used per test concentration and control. The vessels contained a sediment layer (artificial sediment) of approximately 4 cm and a water column (reconstituted water) of 40 cm.

4 applications were made at day 0, 7, 21 and 28.

Applied and measured concentrations :
Nominal concentrations: 8, 25, 80, 250 and 800 µg/l ziram 76 WG corresponding to 6, 19, 61, 189 and 605 µg a.s./l.

Active substance concentrations were monitored in the 19 and the 189 µg a.s./l treatments at 0.5 h, 1, 2 and 7 days after each treatment . Measured concentrations were in the range 87-90 % 30 minutes after application. Measured concentrations were in the range 42-58 % at day 1, 27-46% at day 2 and 12-16% at day 7.

Appplication of 189 µg a.s./l ziram leads to concentrations of thiram in the range 21.9-54 µg/l at day 0 to day 2. less tan 1 µg thiram/l at day 7.

Test conditions :
temperature : 21.8-23.8 °C,
pH : 7.9-8.2
oxygen content : >7.1 mg O_2 /l
total hardness : 2.5 mmol Ca^{2+}
Photoperiod : 16h light and 8 h darkness
Analytical methods : HPLC and MS detection
Findings :
Behavioral observations : 9/10 Fish on the bottom of the test tank and tumbling during swimming were observed at the 605 µg a.s./l from day 1 to 6.

Body wet weight :
Fish weight has been determined at day 0 and 28. body weight of tested groups (6, 19, 61, 189 µg/l) is

Table B.9.2.2-1 : Number of affected fish/dead fish before the applications and at study termination (*)

Nominal concentrations (µg a.s./l)	Day 1	Day 3	Day 7	Day 28	Mean body weight in % of control at study termination
0	0/0	0/0	0/0	0/0	100%
6	0/0	0/0	0/0	0/0	97.4%
19	0/0	0/0	0/0	0/0	89.7%
61	0/0	0/0	0/0	0/0	99.5%
189	0/0	0/0	0/0	0/0	95.4%
605	10/0	10/9	10/10	10/10	-

* : The original report also mentions the clinical symptoms and number of affected organism at each individual day.
* : No change between day 7 and day 28

Conclusions :
NOEC (*Lepomis macrochirus* ,4 appl. in water/sediment syst., 28 day, mortality) : 189 µg a.s./l

B.9.2.7 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

Chronic toxicity of Ziram 76 WG to a population of Daphnia magna after a fourfold application (Memmert, 2001)
Guidelines :
No specific guideline is recommended for this type of test.
GLP :
Yes
Material and Methods :
Test substance : ZIRAM 76 WG formulation containing 75.58% a.s.
Test species : Daphnia magna
Number of organisms, age :
Study was started with populations including 3 ages per test beaker : 8 offspring with an age of less than 24 hours, 5 females with an age of 5-6 days, and 5 females with an age of 12-13 days. Five replicates (test beakers) per treatment and control.
Type of test :
Whole population test in the laboratory
Applied and measured concentrations :
The test item was applied into water on days 0, 7 , 14 and 21 in order to mimick 4-fold applications. The concentrations tested were 0, 1.3, 4.2, 13, 42 and 132 µg/l ZIRAM 76WG. equivalent 0, 1.0, 3.2, 10, 32 and 100 µg a.s/l .

Measured concentrations in water were in the range 88-97 % on day 0. Measured concentrations were in the range 55-81 % at day 1, 17-36% at day 7 just before next application. It was observed that the concentrations rapidly decrease after the last application.

Test conditions :
temperature : 20.4–21.5 °C,
pH : 7.6-8.2
oxygen content : > 7.3 mg/l
total hardness : 2.5 mmol Ca^{2+}

Photoperiod : 16h light and 8 h darkness
Analytical methods : HPLC and MS detection
Findings :
Populations densities were recorded at days 2, 8, 15, 22, 29, 36, 43, 50, 37, 64,and 70 (study termination). The density of the different size classes and the total density of the populations were recorded. Remarkable observations as the development of resting eggs or signs of intoxication were recorded.
Total biomass dry weight has been determined at study termination.

Table B.9.2.7-1 : Total population densities (in % of the control) (*)

Nominal concentrations (µg a.s./l)	Day 8	Day 22	Day 29	Day 50-70	Mean dry weight of the population (in % of control) at study termination
0	100	100	100	100	100%
1	88	101	297	62	168.8%
3.2	104	107	431	63	164.6%
10	89	80	300	81	178.1%
32	7	164	149	104	200.0%
100 (replicates 2,3,5)	Quasi extinction	114	878	87	144.8% (for 5 replicates)
100 (replicates 1,4)	Complete extinction	2 (re-introduced after 4th appl.)	300	151	

*: Some date points of the original report were selected.
** : complete extinction of the daphnids were observed in replicates 1 and 4 of the 100 µg/l treatment. Daphnids were reintroduced on day 22 in these replicates.

Conclusions :
- Important acute effects (mortality and delayed population development) are observed at 32 and 100 µg a.s./l.

- In the 32 µg a./l treatment group, the recovery of the populations was observed at day 22.
- In the 100 µg a.s./l treatment group, quasi extinction or complete extinction was observed after first application. Complete recovery occurs at day 29 (1 week after last appl.) or day 22 (1 day after last appl.), respectively for the replicates with and without re-introduction of individuals. The recovery is correlated with the disappearance of ziram in water.

NOEC (Daphnia magna populations, acute effects of mortality and delayed population development) : 10 µg a.s./l
EAC (Daphnia magna populations, after 4 appl. at 29 days, recovery of populations) : 32 µg a.s./l

B.9.2.9 Effects on sediment dwelling organisms (Annex IIA 8.2.7)

Effects of Ziram 76WG on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system (Memmert, 2001)
Guidelines :

Proposal for a BBA guideline, 1995 Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system
OEC guideline – proposal for a new guideline 219, Feb 2001: sediment-water chironomid toxicity test using spiked water.
GLP :
Yes
Material and Methods :
Test substance : Ziram 76 WG, containing 75.58% ziram
Test species : midges larvae (*Chironomus riparius*)
Number of organisms, weight, length, loading :
25 larvae of first larval stage per concentration.
Type of test :

The vessels contained a sediment layer (artificial sediment) of approximately 3 cm and a water column (reconstituted water) of 12 cm.

Applied and measured concentrations :
The test item was applied once in the water column. Nominal concentrations: 51, 128, 320, 800 and 2000 µg/l Ziram 76 WG corresponding to 38.6, 96.7, 242, 605, and 1512 µg a.s./l.

Active substance concentrations were monitored in the 242 and the 1512 µg a.s./l treatments at 1 h, 7 and 27 days after treatment . The values were corrected for recoveries.

- Measured concentrations in *water* were in the range 107-115% 1 hour after application. Measured concentrations were in the range 7-8% at day 7, 1-6 %at day 27.
- Measured concentrations in *pore water* were in the range 0.4-0.8% 1 hour after application. Measured concentrations were in the range 2.3-3.5% at day 7, 2.2-4.4% at day 27.
- Measured concentrations in *sediment* were in the range 0.014-0.076mg a.s./g sediment for the treatment sample 242 µg a.s/l. They were in the range 0.05-1.04mg a.s./g sediment for the treatment sample 1512 µg a.s./l

Test conditions :
temperature : 19.8-21.4 °C,
pH : 8.0-8.4
oxygen content : >7.1 mg O_2 /l
total hardness : 2 mmol Ca^{2+}
Analytical methods : HPLC and MS detection
Findings :

Table B.9.2.9-1 : emergence ratio

Nominal concentrations (µg a.s./l)	Day 27
0	97%
38.6	91%
96.7	92%
242	97%
605	70%
1512	0%

Conclusions :
NOEC (27 day, *Chironomus* emergence) : 242 µg a.s./l

B.9.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

Risk assessment for fish and daphnia
The following assumptions were made to assess the risk for fish and daphnia :

- The GAP of 2.28 kg a.s./ha, 4 applications at 7 day interval have been considered.
- the impact of repeated applications is considered in both studies
- It can be expected that the metabolites of ziram – mainly thiram- have been formed during the fish and the daphnia studies and are therefore considered in the assessment.

- The NOEC (*Lepomis macrochirus* ,4 appl. in water/sediment syst., 28 day, mortality) : 189 µg a.s./l.
- EAC (Daphnia magna populations, after 4 appl. at 29 days, recovery of populations) : 32 µg a.s./l
- NOEC (Daphnia magna populations, after 4 appl., mortality and delayed population development) : 10 µg a.s./l

The risk assessment for fish and daphnia has demonstrated that acceptable scenarios (GAP of 2.28 kg a.s./ha with 4 applications) can be identified with mitigation methods such as buffer zones of 40-50 m. (NOEC fish and EAC daphnia populations were considered.) (See table B.9.2.16-1)

Risk assessment for algae and Chironomus
The following assumptions were made to assess the risk for algae and Chironomus :

- The GAP of 2.28 kg kg a.s./ha, 1 single application have been considered.
- NOEC (27 day, *Chironomus* emergence) : 242 µg a.s./l .
- It can be expected that the metabolites of ziram – mainly thiram- have been formed during the chironomus study and are therefore considered in the assessment.
-
- EC50 (Selenastrum capricornutum, 96 h, static test) : 0.066 mg a.s./l

The first tier risk assessment for algae and Chironomus has been made for GAP of 2.28 kg a.s./ha, single application. (See table B.9.2.16-2)

The refined risk assessment of the effects on aquatic arthropods and algae after repeated application has been based on the thiram mesocosm. Number of applications (4) and application rate which were evaluated in this mesocosm also reflect the ziram GAP. The evaluation of this mesocosm study is available in the thiram addendum.
Ziram is rapidly degraded to thiram (DT50 ziram, whole system = 0.2-0.3 d). We consider the conservative assumption that 1 mole (305.81 g) ziram is instantaneously converted to 1 mole thiram (240 g).

- the spray drift scenarios are determined according to Ganzelmeier (2000) : orchard early/late growth stages
- application in orchard : 4 applications of 1.79 kg thiram/ha at interval of 7 days (equivalent to 4 applications of 2.28 kg ziram/ ha)

- Aquatic invertebrates and algae : the thiram mesocosm has been performed according to the GAP in orchard/vineyard (see addendum September 2000); EAC : 0.020 mg a.s./l, NOEC : 0.001 mg a.s./l (author of the study report); France and Netherlands propose to take an EAC of **0.010** mg a.s./l.

The risk assessment for aquatic invertebrates and algae has demonstrated that acceptable scenarios (GAP of 2.28 kg a.s./ha with 4 applications) can be identified with mitigation methods such as buffer zones of 30-40 m. (EAC thiram mesocom.) (See table B.9.2.16-3)

Table B.9.2.16-1 : TER for fish and daphnia – 2.28 kg a.s./ha, 4 applications

Toxicity exposure ratios for aquatic organisms					
Appl. rate (kg a.s./ha)	Organism	Distance	TER (early)	TER (late)	Annex VI trigger
2.28 kg a.s./ha, 4 applications	NOEC (*Lepomis macrochirus* , 4 appl. in water/sediment syst., 28 day, mortality) : 189 µg a.s./l	0	**0.2**	**0.2**	10
		3	**0.9**	**1.6**	10
		5	**1.3**	**3.0**	10
		10	**2.1**	**6.9**	10
		15	**4.5**	13.7	10
		20	**9.0**	22.8	10
		30	23.9	46.1	10
		40	47.8	77.7	10
		50	82.9	113.0	10
2.28 kg a.s./ha, 4 applications	EAC (repeated applications , daphnia population) = 32 µg a.s./l	0	**0.0**	**0.0**	10
		3	**0.1**	**0.3**	10
		5	**0.2**	**0.5**	10
		10	**0.4**	**1.2**	10
		15	**0.8**	**2.3**	10
		20	**1.5**	**3.9**	10
		30	**4.0**	**7.8**	10
		40	**8.1**	13.2	10
		50	14.0	19.1	10
2.28 kg a.s./ha, 4 applications	NOEC (repeated applications, daphnia population) = 10 µg a.s./l	0	**0.0**	**0.0**	10
		3	**0.0**	**0.1**	10
		5	**0.1**	**0.2**	10
		10	**0.1**	**0.4**	10
		15	**0.2**	**0.7**	10
		20	**0.5**	**1.2**	10
		30	**1.3**	**2.4**	10
		40	**2.5**	**4.1**	10
		50	**4.4**	**6.0**	10

Table B.9.2.16-2 : TER for algae and chironomus – 2.28 kg a.s./ha, single application

Toxicity exposure ratios for aquatic organisms					
Appl. rate (g a.s./ha)	Organism	Distance	TER (early)	TER (late)	Annex VI trigger
2280	EC50 algae = 0.066 mg a.s./l	0	**0.1**	**0.1**	10
		3	**0.3**	**0.6**	10
		5	**0.4**	**1.0**	10
		10	**0.7**	**2.4**	10
		15	**1.6**	**4.8**	10
		20	**3.1**	**8.0**	10
		30	**8.4**	16.1	10
		40	16.7	27.1	10
		50	28.9	39.5	10
2280	NOEC Chironomus 0.242 mg a.s./l	0	**0.3**	**0.3**	10
		3	**1.1**	**2.0**	10
		5	**1.6**	**3.8**	10

		10	**2.7**	**8.8**	10
		15	**5.7**	17.6	10
		20	11.5	29.2	10
		30	30.6	59.0	10
		40	61.2	99.5	10
		50	106.1	144.7	10

Table B.9.2.16-3 : TER related to the application in orchard : 4 applications of 2.28kg ziram/ha at interval of 7 days

Toxicity exposure ratios for aquatic organisms					
Appl. rate (kg a.s./ha)	Organism	Distance	TER (early)	TER (late)	Annex VI trigger
1.79 kg thiram equivalent to 2.28 kg ziram	EAC Mesocosm = 0.010 mg a.s./l	0	**0.0**	**0.0**	3
		3	**0.1**	**0.1**	3
		5	**0.1**	**0.2**	3
		10	**0.1**	**0.5**	3
		15	**0.3**	**0.9**	3
		20	**0.6**	**1.5**	3
		30	**1.6**	3.1	3
		40	3.2	5.2	3
		50	5.6	7.6	3

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3.1)

B.9.5 Effects on other arthropods species (Annex IIA 8.3.2; Annex IIIA 10.5)

B.9.5.2 Effects of the formulations on non-target terrestrial arthropods (laboratory, semi-field tests) Annex IIIA 10.5.1)

Ziram 76WG: toxicity to the green lacewing, Chrysoperla carnea Steph. (Neuroptera, Chrysopidae) in the laboratory (Kemmeter, 2001)
Guidelines :
Guidelines of Bigler (1988) and Vogt et al. 2000) and the guidance document for regulatory testing procedures for pesticides with non-target arthropods (Barrett et al., 1994)
GLP :
yes
Material and Methods :
Test substance : Ziram 76 WG, containing 76.96% ziram

Test species : Chrysoperla carnea
Number of organisms : 60 larvae per treatment
Type of test : lab test
Exposure route :
Larvae of Chrysoperla were exposed to the dried layer of the test substance freshly applied on glass plates. Final pre-imaginal mortality was calculated after the emergence of the adults. The reproduction performance of the adults was examined over 7 days (egg count and emergence of larvae)

Test conditions :

Temperature 22-29°C
Relative humidity : 54-78%
Long day conditions : 16 hlight/8 h darkness
Light intensity : 2870-3340 lux

Findings :
Table B.9.5.2-1 : Effect of Ziram 76WG on *Chrysoperla carnea* – laboratory

	Preimaginal Mortality [%]	Fecundity (number of fertile eggs/female/d)
Water control	6.67	47.59
Toxic standard (dimethoate)	78.34	Not determined
4 kg formulation/ha	25.00	47.66
8 kg formulation/ha	13.33	42.09
	Corrected preimaginal mortality [%]	
4 kg formulation/ha	19.64	
8 kg formulation/ha	7.14	

Conclusion :
Based on these results no adverse effects of Ziram 76 WG on pre-imaginal mortality and reproduction performance if Chrysoperla carnea were observed under laboratory conditions at rate of 4 and 8 kg formulation/ha.

B.9.5.3 Effects of the formulations on non-target terrestrial arthropods (field tests) Annex IIIA 10.5.2)

Effects of Ziram 76% WG on predatory mites (Acari: Phytoseiidae) in a pome fruit orchard in France (Müther, 2000)
The effects of the fungicide Ziram 76 WG on the predatory mites was assessed by conducting a field trial in a pome fruit orchard. The test site was located in Southern France (Rhone Valley). The only predatory mite present at the test site was Neoseiulus californicus
Conclusion :
We agree with the conclusion of the author that 'Due to lack of statistically significant effect and not fulfilled validity criteria, this study should not be used for classification of side effects of Ziram 76WG on predatory mites in pome fruits.'

B.9.5.4 Summary of effects, exposure and risk assessment for non-target terrestrial arthropods

Argumentation in defence of 4 application (UCB, Cerexagri, FMC, 2001)
The notifier submitted an argumentation indicating that predatory mites would not be at risk, even in the case of 4 applications/season. The predatory mites would not be present in the early season around flowering. The definition of GAP (right timing of the applications) should be made at MS level.