Thiram

Addendum to the monograph
November 2002

B.9 Ecotoxicology

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

Study to generate seed samples and seedlings for determination of the loss of detectable thiram from drilled wheat seed, UK 2001 (Pritchard, 2001c)
GLP :
Yes
Material and Methods :
Test Substance: THIRAFLO 480 g/l FS
Experimental design :
Three field trials were drilled in the early spring (mid March) of 2001 using seed treated with thiram for the purpose of providing seedling samples, to determine how much thiram remains on the growing seedling just after emergence. Samples were taken from the plots at each site at Zadoks growth stage 10 (first leaf through coleoptile) which occurred 24-28 days after drilling.
A second series of plots, were drilled using the treated seed in late spring (mid April) of 2001 to represent a later sown crop. These were sampled at Zadoks growth stage 10 which occurred 19-27 days after drilling.

Soil properties (Silty clay, organic loamy sand, sandy loam) and weather data are reported in the study.
Findings :
Table B.9.1-1 : Thiram loading on spring wheat seedlings

	Data range	median
Seed loading (mg a.s./kg seed)	749-769	756
Seed loading (µg a.s./seed)	36.4-37.8	37.0
Seedling loading (mg a.s./kg seedling)	0.79-86.9	29.8
Seedling loading (µg a.s./seedling)	0.1-11.5	3.8
a.s. loading in terms of weight of seed originally used for planting (mg a.s./kg seed)	2.6-133	34.2

Conclusions :
The a.s. level in cereal seedling is 0.1 to 11% of the a.s. level in treated seeds

Study to generate seed and seedling samples from thiram treated, broadcast on to the soil (Pritchard, 2001b)
GLP :
Yes
Material and Methods :
Test Substance: THIRAFLO 480 g/l FS
Experimental design :
Spring wheat was treated with a nominal 600 ppm thiram. Three field trials were established in the early spring of mid March 2001 to provide samples of seed/seedlings that have been broadcast on to the soil surface and exposed to natural weather conditions for specified periods of time. The broadcast was repeated on two later occasions in the spring. Samples of 200 seedlings were intended to be taken 0, 1, 3, 7, 14 and 28 days after broadcasting but in practice, despite the provision of protection in the form of netting this was not possible as much as the seed disappeared.

Findings :
Table B.9.1-2 : Rate of degradation of thiram on broadcast seed in three UK fields

	seed	Seed/seedling				
		At day 0	At day 1	At day 3	At day 7	At day 14
Field 1						
loading (mg a.s./kg seedling)	769	646	369	268	194	62.2
loading (µg a.s./seedling)	36.4	34.8	22.1	17.8	11.5	4.6
Field 2						
loading (mg a.s./kg seedling)	756	607	463	432	358	
loading (µg a.s./seedling)	37.8	32.8	27.1	25.4	20.2	
Field 3						
loading (mg a.s./kg seedling)	749	423	194	122	58.8	6.2
loading (µg a.s./seedling)	37.0	25.2	13.1	9.6	3.9	0.4

The experiment has been repeated at 2 sowing dates for each site. Results of both series were similar Only the 'longest' series of data has been reported in this table.

Conclusion :
DT_{50} of thiram on seeds is around 3 days.

Study to determine the amount of thiram treated seed and seedlings when treated seed remains uncovered at drilling, UK 2001 (Pritchard, 2001a)
GLP :
Yes
Material and Methods :
Test Substance: THIRAFLO 480 g/l FS
Experimental design :
When seed is precision drilled a certain amount of seed is left on the soil surface. Previous studies have suggested levels of up to 100 seeds per metre^2. This uncovered seed may be taken by birds or small mammals, leading to ingestion of quantities of the seed treatment chemical. This experiment was designed to simulate this effect and to monitor the rate at which the uncovered seed disappeared.

Immediately after broadcasting the seed 10 X 1 m^2 quadrats were permanently marked at random positions within the plot. At the required time period following drilling the number of seeds/plants remaining within each quadrat was recorded.

Soil properties and weather data are reported in the study.
Findings :
Table B.9.1-3 : total number of seeds, plants present in 10 quadrats

Days after broadcasting	Field 1	Field 2	Field 3
0	809	987	1030
3	442	670	522
7	42	0	4
14	0	-	6
21	-	-	0
28	-	-	-

Conclusion :
Initial densities of approximately 100 seeds m^2 were reduced by 49 to 32% within three days and almost completely disappeared within seven days. There were no great differences in rate of disappearance between the three sites.

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

Assessment of the acute and short term risk according to the new guidance document
The acute and short term risk has been reconsidered according to the new guidance document and the new information available. This assessment confirms the absence of acute and short term risk to birds.

The following assumptions can be made :

- formulation thiram 480 g/l, application rate in cereals of 0.125 l/100 kg seeds is equivalent to a loading of **600 mg a.s./kg seed.**
- Birds have access to seeds (DT_{50} of thiram on seeds is 3 days, TWA residue on seed for a 7-day availability period is equivalent to **50% of the initial residue**) and young seedlings (0.1 to 11% of the initial seed loading)

- The guidance document on risk assessment for birds and mammals (SANCO/4145/2000) has been considered.
- LD_{50} is **2000** mg/kg bw
- The LC_{50} > 4423 mg/kg food is equivalent to **> 947** mg a.s./kg bw/d (birds weighing 70g are consuming 15 g food/d, data from the original study)
- The NOEC of 500 mg/kg food is equivalent to **37.5** mg a.s./kg bw/d (birds weighing 200g are consuming 15 g food/d, data from the original study)

Toxicity exposure ratios for birds				
	Organism	Time scale	TER	Annex VI trigger
600 mg a.s./kg seeds	granivorous bird	Acute	17.54	10
	granivorous bird	short term	8.32	10
	granivorous bird	long term	0.33	5

Acute and short term risk are acceptable for birds eating seeds (TER > 10 or close to 10 for birds consuming 100% treated seeds). The risk to birds eating seedlings is negligible.

The long term TER (0.33) calculated according to the new guidance document should not be considered in the risk assessment (exaggerated worst case). According to the guidance document, it should be checked whether short term exposure is likely to result in long-term effects. This evaluation is made in the following paragraph.

Further assessment of the risk to breeding birds (spring exposure)
The risk assessment to birds consuming treated seeds in spring has been completed.

- **The reversibility of the effects on the reproduction function** has been demonstrated in the laboratory bobwhite reproduction study (Beavers, 1995). No effects on reproduction parameters were observed in the '2500 ppm withdrawal' group. In this group, thiram administration had been interrupted prior to the egg production period.

- The duration and level of exposure
- The duration of possible exposure to thiram is around 7 days for seeds (Pritchard, 2001a).
- The level of thiram in young seedlings is much lower than in seeds (Pritchard, 2001c). Risk form the consumption of the crop at this stage is negligible.
- The degradation rate of the a.s. on seeds is rapid (DT_{50} around 3 days) (Pritchard, 2001b). The initial a.s. level on seeds (600 mg/ kg) is quickly reduced within a few days below the NOEC of the reproduction study (500 mg/kg food)

- **Overlap of breeding period and sowing**: there is no significant overlap for autumn sown cereals and only very limited overlap for certain spring sown crops. Cereal sowing dates (February-March for spring barley, November-early march for spring wheat) and breeding periods of birds (April-November; precise periods are given for several species) have been reported by the notifier. (The Cereals handbook, (NIAB)), (CSL, 1998 Birds and Farming, Buxton et al.)

- **Additional risk mitigations factors**
The 'Evaluation of the risk to birds from the spring sowing of seed treated with thiram risk assessment' (Pritchard, 2001d) also mentions the following risk mitigations factors :

- warning phrases appearing on products labels and bags of treated seeds (do not broadcast treated seeds, treated seed may be harmful to game and wildlife, bury all spillage) would lay a statutory obligation on users to bury spillages, which represent the greatest potential of exposure.
- A limited part of the fields are treated with thiram (Within EU, UK is the most important market for thiram treated seeds; 10% of the UK wheat is treated with thiram, 2% of barley is treated with thiram)
- Over 90% of UK small grain cereal is automn sown.
- The repellency of the a.s. (The repellency studies have been assessed in the previous addendum)

The risk to birds consuming treated seeds sown in spring is acceptable.

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

B.9.2.1 Acute toxicity of the active substance and metabolites, degradation or reactions products to fish (Annex IIA 8.2.1)

Acute toxicity of thiram technical to rainbouw trout (*Oncorhynchus mykiss*) in a 96-hour static test (Peither, 2001)
Guidelines :
OECD N° 203
GLP :
Yes
Material and Methods :
Test substance : Thiram Technical (Purity: 99,01%)
Test species : Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, age : 7 fishes per 15 l of test medium (mean body length : 4.5 ±0.33 cm, mean wet weight 1.1 ±0.33 g,
Type of test : Acute toxicity test (96 hours, static)
Applied and measured concentrations : 0.0032, 0.010, 0.032, 0.32 and 1.0 mg/l. Concentrations were measured at 0, 48 and 96 h. Measured concentrations in the range 89-106% at t 0; At 0.032, 0.10 and 0.32 mg/l nominal concentrations the concentrations had decreased at 7, 12 and 25% of nominal after 48 hours.

Due to the fast degradation of the a.s., the reported results are related to the initially measured test concentrations.
Test conditions :
temperature : 13-14 °C,
pH : 7.6-7.9
oxygen content : >8.8 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 : tumbling during swimming observed at 0.031-0.30 mg/l
Endpoints :
LC_{50} (96h) : 0.046 mg a.s./l (95% confidence limits : 0.028-0.077)
NOEC (96 h) : 0.011 mg a.s./l
Conclusions :
Thiram is very toxic to rainbow trout.

Fish toxicity (Bayer AG, 1978)
Guidelines :
OECD N° 203
GLP :
No
Material and Methods :
Test substance : thiram technical, (purity : 96.7%)
Test species : Leuciscus idus melanotus
Number of organisms, weight, length, age :
10 fishes per 15 l of test medium (body length: 6 cm - body weight: ± 1.5 g).
Type of test :
Acute static toxicity test, 96 hours
Applied and measured concentrations :
Nominal concentrations: 0.0032, 0.010, 0.032, 0.10, 0.32 and 1.0 mg/l and a solvent control. Measured concentration of 85% after 96 h (not in accordance with the rapid degradation of the a.s. in other studies.
Test conditions :
temperature : 22 °C,
pH : 7.3-7.7
oxygen content : > 7 mg O_2 /l
total hardness : 15°dH equivalent to ca 2.13mmole Ca^{2+}/l and ca 0.6 mmole Mg^{2+}/l.
Photoperiod : 12 h light and 12 h darkness
Analytical methods : G.E. Keppel, J.A.O.A.C. 54, 528 (1971)
Conclusions :
Study of low quality. LC_{50} (96h) : 1.2 mg/l (95% confidence limits : 1.1-1.3 mg/l)

Acute toxicity of Thiurad® to Fathead Minnow *(Pimephales promelas)* (Chole, 1983)
Guidelines :
- Standard Methods for Examination of Water and Wastewater. American Public Health Association. 1975. 14th ed. Washington, DC. 1193 p.
- Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Committee on Methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan, Chairman). 1975. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April 1975, 61 p.

GLP :
Yes
Material and Methods :
Test substance : Thiurad® (thiram Monsanto origin) (purity > 96%)
Test species : Fathead Minnow *(Pimephales promelas)*
Number of organisms, weight, length, age :
10 fish / 15 l test medium (mean standard length of 18 (± 1.4) mm - mean weight of 0.10 (± 0.027) g.
Type of test :
Acute static toxicity test, 96 hours
Applied and measured concentrations :
Nominal concentrations: 0, 0.10, 0.18, 0.32, 0.56, 1.0 mg/l and a solvent control. Concentrations were not measured.
Test conditions :
temperature : 21-22 °C,
pH : 8.1-8.3
oxygen content : > 8.2 mg O_2 /l
total hardness : 255 mg/l $CaCO_3$
Photoperiod : 16 h light and 8 h darkness
Analytical methods : not reported
Conclusions :
LC_{50} (96h) : 0.27 mg/l (0.21-0.34 mg/l - 95% confidence limits)
NOEC : 0.10 mg/l

Acute toxicity of Thiurad® to Bluegill Sunfish *(Lepomis macrochirus)* (Forbis, 1983)
Guidelines :
- Standard Methods for Examination of Water and Wastewater. American Public Health Association. 1975. 14th ed. Washington, DC. 1193 p.
- Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Committee on Methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan, Chairman). 1975. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April 1975, 61 p.

GLP :
Yes
Material and Methods :
Test substance : Thiurad® (thiram Monsanto origin) (purity > 96%)
Test species : Bluegill Sunfish *(Lepomis macrochirus)*
Number of organisms, weight, length, age :
10 fish / 15 l medium (mean standard length of 16 (± 2.1) mm - mean weight of 0.09 (± 0.04) g.
Type of test :
Acute static toxicity test, 96 hours
Applied and measured concentrations :
Nominal concentrations: 0.056, 0.10, 0.18, 0.32 and 0.56 mg/l and a solvent control. Concentrations were not measured.
Test conditions :
temperature : 21-22 °C,
pH : 7.8-8.1
oxygen content : > 7.3 mg O_2 /l
total hardness : 255 mg/l $CaCO_3$
Photoperiod : 16 h light and 8 h darkness
Analytical methods : not reported
Conclusions :
LC_{50} (96h) : 0.13 mg/l (0.076-0.20 mg/l - 95% confidence limits)
NOEC : 0.056 mg/l

B.9.2.2 Fish chronic toxicity test (Annex IIA 8.2.2)

Thiram is rapidly degraded in water with some dissipation to the sediment. Chronic effects would be predominantly due to repeated exposure.
The protocol of the study has been adapted to take these points into consideration.

Sublethal effects of Thiram 80 WG to Rainbow trout *(Oncorhynchus mykiss)* after a fourfold application to a water-sediment system
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 : Thiram 80 WG, containing 81.20% thiram
Test species : Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading :
10 fishes per concentration. Initial loading rate was ± 0.1 g/l; at the end ± 0.37 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. The fish of concentrations 0, 12, 20 µg/l were observed for 2 additional weeks (from day 28 to 42)

Applied and measured concentrations :
Nominal concentrations: 15, 25, 39, 63 and 100 µg/l Thiram 80 WG corresponding to 12, 20, 31, 50 and 80 µg a.s./l.
Active substance concentrations were monitored in the 20 and the 50 µg/l treatments at 0.5, 24 and 48 hours after each treatment . Measured concentrations were in the range 72-90% 30 minutes after application. Measured concentrations were in the range 3-18 % at t 24 h. Measured concentrations were in the range 0-13% % at t 48 h.

Test conditions :
temperature : 13.7-16.2 °C,
pH : 7.7-8.0
oxygen content : >7.4 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 : Fish lying on side or back on the bottom and tumbling during swimming observed at 31 and 50 µg/l
Table B.9.2.2-1 : Number of dead fish before the applications and at study termination (*)

Nominal concentrations (µg a.s./l)	Day 6	Day 13	Day 20	Day 27	Day 42
0	0%	0%	0%	0%	0%
12	0%	0%	0%	0%	0%
20	0%	10%	10%	10%	10%
31	0%	0%	0%	30%	No post-treatment
50	0%	0%	10%	40%	
80	60%	100% (at d 8)	100%	100%	

* : The original report also mentions the clinical symptoms and number of affected fish at each individual day.

Body wet weight :
Fish weight has been determined at day 0, 28 and 42.

Table B.9.2.2-2 : % growth

Nominal concentrations (µg a.s./l)	% growth (wt day 28/ wt day 0)	% growth (wt day 42/ wt day 0)
0	252	364
12	222	346
20	207	318
31	140	-
50	117	-
80	-	-

Table B.9.2.2-3 : mean growth rate

Nominal concentrations (µg a.s./l)	Mean growth rate		
	Day 0-28	Day 28-42	Day 0-42
0	3.23	2.34	2.93
12	2.84	3.17	2.95
20	2.57 (*)	3.09	2.74
31	1.20 (*)	n.a.	n.a.
50	0.55 (*)	n.a.	n.a.
80	-	n.a.	n.a.

(*) : statistically significant

Conclusions :
NOEC (42 day, mortality) : 20 µg a.s./l (one fish died at day 9 at 20 µg/l, this would not be related to treatment)

NOEC (42 day, mean growth rate on 0-28 day period) : 12 µg a.s./l
(The growth rate of 2.84 at 12 µg a.s./l is not statistically different from control; the growth rate further recovers at day 28-42.)

B.9.2.11 Acute toxicity of the preparations (Annex IIIA 10.2.1)

Acute toxicity of Thiram 80WG to rainbow trout *(Oncorhynchus mykiss)* in a static water-sediment system (Memmert, 2001)
Guidelines :
OECD N° 203 ; with some modifications to mimic more realistic use conditions : performed in presence of sediment
GLP :
No
Material and Methods :
Test substance : Thiram 80 WG, containing 81.20% thiram
Test species : Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, age :
7 fishes per concentration (mean body length: 4.7 ± 0.26 cm (Mean ± SD) - mean body wet weight: 1.06 ± 0.24 g (Mean ± SD)).
Type of test :
Acute static toxicity test, 96 hours, prolonged up to 11 days
The water-sediment systems were prepared in glass aquaria in a temperature-controlled laboratory.

One aquarium with a water volume of 30.3 liters was used per test concentration and control. The aquaria contained a sediment layer (artificial sediment) of approximately 3 cm and a water column (reconstituted water) of 24 cm.
Applied and measured concentrations :
Nominal concentrations: 25, 125 and 625 µg/l of the formulation Thiram 80 WG (corresponding to approximately 20, 100 and 500 µg/l of the active ingredient Thiram).
Test conditions :
temperature : 14-15 °C,
pH : 7.9-8.0
oxygen content : >8.0 mg O_2 /l
total hardness : 2.5 mmol Ca^{2+}
Photoperiod : 16h light and 8 h darkness
Analytical methods : HPLC and MS detection
Conclusions :
LC_{50} (96h) : 111 µg/l thiram 80WG, corresponding to 89 µg a.s./l (95% confidence limits : 28-451 µg/l)
NOEC (96 h) : 25 µg/l thiram 80WG, corresponding to 20 µg a.s./l

Acute toxicity of Thiram Granuflo (i.a. 80% p/p) in fish *(Cyprinus carpio)* (Bossotto, 2001)
Guidelines :
OECD N° 203
GLP :
yes
Material and Methods :
Test substance : THIRAM GRANUFLO, containing 80 % thiram
Test species : Carp *(Cyprinus carpio)*
Number of organisms, weight, length, age :
10 fishes per concentration (length: 3.0 ± 1.0 cm).
Type of test :
Acute toxicity test, 96 hours, daily renewal of the test solutions
Applied and measured concentrations :
Nominal concentrations: 0.1, 0.2, 0.4, 0.8, 1 mg/l of the formulation THIRAM GRANUFLO.
Measured concentrations were in the range 75-99%
Test conditions :
temperature : 20-22 °C,
pH : 6.9-7.3
oxygen content : 7.0-7.3 mg O_2 /l
total hardness : 60 mg $CaCO_3$/l
Photoperiod : 12 h light and 12 h darkness
Analytical methods : HPLC detection
Conclusions :
LC_{50} (96h) : 0.25 mg/l THIRAM GRANUFLO, corresponding to 0.20 mg a.s./l (95% confidence limits : 0.13-0.39 mg/l)

B.9.2.15 Summary of effects to water organisms - Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

The following points have been addressed :
Acute fish toxicity study
Chronic fish toxicity study, evaluation of the risk to fish after repeated application.
The risk of the active and major metabolites in the water/sediment study to sediment dwelling organisms is addressed in the fate part of the addendum.

Table B.9.2.15-1 : Summary of effects of thiram to water organisms – New studies

Test species	Test system	Duration of exposure	Results (mg a.s./l)	References
Oncorhynchus mykiss	Static test, a.s.	96 hours	LC_{50} = 0.046 mg a.s./l NOEC = 0.011 mg a.s./l	Peither, 2000
Pimephales promelas	Static test, a.s.	96 hours	LC_{50} = 0.27 mg a.s./l NOEC = 0.10 mg a.s./l	Chole, 1983
Lepomis macrochirus	Static test, a.s.	96 hours	LC_{50} = 0.13 mg a.s./l NOEC = 0.056 mg a.s./l	Forbis, 1983
Oncorhynchus mykiss	Static test, 80WG, 4 applications at 7 day interval Water/ sediment system	42 days	NOEC (42 d, mortality) = 0.020 mg a.s./l NOEC (42 d, growth rate) = **0.012** mg a.s./l	Memmert, 2001
Oncorhynchus mykiss	Static test, 80WG, Water/ sediment system	96 hours, prolonged up to 11 days	LC_{50} = **0.089** mg a.s./l NOEC = 0.020 mg a.s./l	Memmert, 2001
Cyprinus carpio	Semi-static test (daily renewal of test solution), 80WG	96 hours	LC_{50} = 0.200 mg a.s./l	Bossotto, 2001

Exposure of water surface is mainly due to spray drift resulting from application in orchard and vineyard . We assumed that the contribution of other routes of contamination is small in comparison with the drift .

The following assumptions were made to assess the acute risk for water organisms :

- water layer : 30 cm
- Repeated applications were included in the studies. Therefore a.s. degradation has not to be taken into consideration in the TER calculations

- the spray drift scenarios are determined according to Ganzelmeier (2000) : orchard early/late growth stages
- application in orchard : 4 applications of 2.4 kg a.s./ha at interval of 7 days
- application in vineyard : 3 applications of 3.2 kg a.s./ha at interval of 7 days

The risk evaluation has been made with the higher tier data :

- Fish : a static test in presence of sediment allows to investigate the acute effects : LC_{50} : **0.089** mg a.s./l
- Fish : a laboratory study with repeated applications in presence of sediment allows to investigate the long term effects : NOEC (42 days, growth rate) = **0.012** mg a.s./l
- Aquatic invertebrates and algae : a 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.

Table B.9.2.15-2 : TER related to the application in orchard : 4 applications of 2.4 kg a.s./ha at interval of 7 days

Toxicity exposure ratios for aquatic organisms					
Appl. rate (g a.s./ha)	Organism	Distance	TER (early)	TER (late)	Annex VI trigger
2400	LC50 fish, in presence of sediment = 0.089 mg a.s./l	0	**0.1**	**0.1**	100
		3	**0.4**	**0.7**	100
		5	**0.6**	**1.3**	100
		10	**0.9**	**3.1**	100
		15	**2.0**	**6.1**	100
		20	**4.0**	**10.2**	100
		30	**10.7**	**20.6**	100
		40	**21.4**	**34.8**	100
		50	**37.1**	**50.6**	100
2400	NOEC fish study with repeated applications = 0.012 mg a.s./l	0	**0.0**	**0.0**	10
		3	**0.1**	**0.1**	10
		5	**0.1**	**0.2**	10
		10	**0.1**	**0.4**	10
		15	**0.3**	**0.8**	10
		20	**0.5**	**1.4**	10
		30	**1.4**	**2.8**	10
		40	**2.9**	**4.7**	10
		50	**5.0**	**6.8**	10
2400	EAC Mesocosm = 0.010 mg a.s./l	0	**0.0**	**0.0**	3
		3	**0.0**	**0.1**	3
		5	**0.1**	**0.1**	3
		10	**0.1**	**0.3**	3
		15	**0.2**	**0.7**	3
		20	**0.5**	**1.1**	3
		30	**1.2**	**2.3**	3
		40	**2.4**	3.9	3
		50	4.2	5.7	3

Table B.9.2.15-3 : TER related to the application in vineyard : 3 applications of 3.2 kg a.s./ha at interval of 7 days

Toxicity exposure ratios for aquatic organisms					
Appl. rate (g a.s./ha)	Organism	Distance	TER (early)	TER (late)	Annex VI trigger
3200	LC_{50} fish, in presence of sediment = 0.089 mg a.s./l	0	**0.1**	**0.1**	100
		3	**3.1**	**1.0**	100
		5	**7.1**	**2.3**	100
		10	**21.4**	**6.8**	100
		15	**41.7**	**12.8**	100
		20	**64.1**	**19.8**	100
		30	119.1	**37.9**	100
		40	208.4	**59.5**	100
		50	277.9	**83.4**	100
3200	NOEC fish study with repeated applications = 0.012 mg a.s./l	0	**0.0**	**0.0**	10
		3	**0.4**	**0.1**	10
		5	**1.0**	**0.3**	10
		10	**2.9**	**0.9**	10
		15	**5.6**	**1.7**	10
		20	**8.7**	**2.7**	10
		30	16.1	**5.1**	10
		40	28.1	**8.0**	10
		50	37.5	11.3	10
3200	EAC Mesocosm = 0.010 mg a.s./l	0	**0.0**	**0.0**	3
		3	**0.3**	**0.1**	3
		5	**0.8**	**0.3**	3
		10	**2.4**	**0.8**	3
		15	4.7	**1.4**	3
		20	7.2	**2.2**	3
		30	13.4	4.3	3
		40	23.4	6.7	3
		50	31.3	9.4	3

A refined risk assessment has been performed for the main foliar uses (orchards and vineyards). The risk is not completely acceptable for the aquatic organisms when applying the classical assumptions. Further mitigations measures should be applied.

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

Thiram: Palatability of treated seed to wood mice (*Apodemus sylvaticus*) (Thompson, 2001a, 2001b)
Guidelines :
No specific guideline
GLP

Yes

Material and Methods :

Two studies with similar protocols were submitted by UCB and Uniroyal.Test substances were respectively Flowsan FS treated maize (maize loading : 1275 mg a.s./kg seed), Thiraflo treated wheat (wheat loading : 527 mg a.s./kg seed)

Test species : Wood mice from breeding colonies, originating from wild caught mice (*Apodemus sylvaticus*), minimum 6 weeks old.

Type of test :

Following a pre-treatment period, 4 replicates of each treatment and two replicates of each control treatment were performed on day 0 (deprived /not deprived X normal diet available/normal diet not available)

Half of each test and control treatment were then offered either a choice in space (treated seed and normal diet available at various places in the cage) or a choice in time (treated seed available during 6 hours and normal diet available the rest of the day) over the following 5 days.

Food consumption was monitored by recording the amount of seed, normal diet available to mice at days –9, -7, 0, 1 to 5. Spilt or hoarded seed were also weighed.

Bodyweight were recorded at days –14, -9, -7, -2, 0, and +6.

	Number of mice	Test day (day 0)		Follow on test (during 5 days)	
				6 hour of night period	Remaining 18 hours
Treatment groups					
1A	2	5 hour deprivation period	Treated seed + normal diet	Treated seed + normal diet	Normal diet only
1B	2			Treated seed only	Normal diet only
2A	2		Treated seed only	Treated seed + normal diet	Normal diet only
2B	2			Treated seed only	Normal diet only
3A	2	No deprivation period	Treated seed + normal diet	Treated seed + normal diet	Normal diet only
3B	2			Treated seed only	Normal diet only
4A	2		Treated seed only	Treated seed + normal diet	Normal diet only
4B	2			Treated seed only	Normal diet only
Control groups					
5A	1	5 hour deprivation period	untreated seed + normal diet	Untreated seed + normal diet	Normal diet only
5B	1			Untreated seed only	Normal diet only
6A	1		Untreated seed only	Untreated seed + normal diet	Normal diet only
6B	1			Untreated seed only	Normal diet only
7A	1	No deprivation period	Untreated seed + normal diet	Untreated seed + normal diet	Normal diet only
7B	1			Untreated seed only	Normal diet only
8A	1		Untreated seed only	Untreated seed + normal diet	Normal diet only
8B	1			Untreated seed only	Normal diet only

Findings and conclusions:
Similar conclusions can be taken for the wheat and maize studies :

- All mice remained in good health and no abnormal behaviour nor clinal signs were noted.
- Comparison of seed consumption by the various treatment groups does not reveal a clear repellent effect of thiram treated seed. Consumption of up to 3.7 g treated seed/day/animal was noted (figures were similar for maize and wheat).
- Bodyweight changes were variable and not treatment related.
- Under the conditions of the studies both seed varieties were palatable and were consumed irrespective of whether another food source was available or not.

Assessment of the acute and short term risk according to the new guidance document
The acute risk has been reconsidered according to the new guidance document and the new information available. This assessment confirms the absence of acute risk to mammals.

The following assumptions can be made :

- formulation thiram 480 g/l, application rate in cereals of 0.125 l/100 kg seeds is equivalent to a loading of **600 mg a.s./kg seed.**
- Small mammals have access to seeds (DT_{50} of thiram on seeds is 3 days, TWA residue on seed for a 7-day availability period is equivalent to **50% of the initial residue**).
- The guidance document on risk assessment for birds and mammals (SANCO/4145/2000) has been considered.
- LD_{50} rat : **1800** mg a.s./kg
- Two generation reproductive toxicity in rat : NOAEL = **1.5** mg/kg bw/d or 30 ppm

Toxicity exposure ratios for mammals				
	Organism	Time scale	TER	Annex VI trigger
600 mg a.s./kg seed.	granivorous mammal	acute	16.67	10
	granivorous mammal	long term	0.01	5

Further assessment of the short term risk to mammals
The long term TER (0.01) calculated according to the new guidance document should not be considered in the risk assessment (exaggerated worst case) . According to the guidance document, it should be checked whether short term exposure is likely to result in long-term effects.

Long term exposure can be excluded because :
- The duration of possible exposure to thiram is around 7 days for seeds (Pritchard, 2001a).
- The level of thiram in young seedlings is much lower than in seeds (Pritchard, 2001c). Risk form the consumption of the crop at this stage is negligible.
-The degradation rate of the a.s. on seeds is rapid (DT_{50} around 3 days) (Pritchard, 2001b).

- The palatability studies with maize and wheat have shown that the consumption of seeds treated at normal application rate during 6 days has no impact on the mice (bodyweight changes were not treatment-related, no clinical signs)

The 'Evaluation of the risk to birds from the spring sowing of seed treated with thiram risk assessment' (Pritchard, 2001d) also mentions the following risk mitigations factors. These factors are also relevant for small mammals :

- warning phrases appearing on products labels and bags of treated seeds (do not broadcast treated seeds, treated seed may be harmful to game and wildlife, bury all spillage) would lay a statutory obligation on users to bury spillages, which represent the greatest potential of exposure.
- A limited part of the fields are treated with thiram (Within EU, UK is the most important market for thiram treated seeds; 10% of the UK wheat is treated with thiram, 2% of barley is treated with thiram)

The risk to mammals consuming treated seeds is acceptable.

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

Toxicity of Thiram 80WG to larvae of the ladybird *Coccinella septempunctata* L. under laboratory conditions. (schuld, 2001)

Guidelines :

- BBA VI, 23-2.1.5 (1989), IOBC Draft (Schmuck et al. 2000),
- ESCORT recommendation (Barrett et al. 1994)

GLP :

Yes

Material and Methods :

Test substance : Thiram 80WG (82.59% analysed)

Test species : Coccinella septempunctata L. (Coleoptera: Coccinellidae)

stage: larvae (L_2, 3-5 days after hatch)

Number of organisms : 50 larvae per treatment.

Type of test : Exposure of *C. septempunctata* L. to dry residues of Thiram 80 WG on glass plates.

Applied concentrations :

Control (deionized water), 2 test concentrations (3 kg product/ha (= 2.4 kg a.i./ha)
6 kg product/ha (= 4.8 kg a.i./ha)) and toxic standard (Afugan 30 EC, 0.01 l product/ha) all applied at a volume equivalent to 200 l/ha.

Test conditions :

temperature:	22-26°C
relative humidity:	70-82%
lightening intensity:	1100 lx
photoperiod:	16 hours light: 8 hours dark

Findings :

Table B.9.5.2-1 : Effect of Thiram 80WG on *Coccinella septempunctata* (lab test)

Application rate of Thiram 80WG	control	3 kg/ha (1 x full rate)	6 kg/ha (2 x full rate)
Mortality (%)	10	90*	82*
Corrected mortality (according to Schneider-Orelli after 19 days) M (%)	-	**89**	**80**

* statistically significantly different ($p \leq 0.05$)

Conclusion :

E (mortality) : 89 and 80 % at 2.4 and 4.8 kg a.s./ha (max. 19 days until hatch) and anomalous behaviour of the treated larvae (i.e. effects on the moulting) were observed in both test item groups compared to the control group.

Thiram 80 WG: An extended laboratory study to evaluate the effects on the Aphid Parasitoid, *Aphidius rhopalosiphi* DeStefani-Perez (Hymenoptera, Braconidae). (Schuld, 2001)

Guidelines :

POLGAR (1988), MEAD-BRIGGS (1996) and ESCORT I Guidance Document (BARRETT et al. 1994)
the guideline of the ring testing group (MEAD-BRIGGS et al., 2000)

GLP :

Yes

Material and Methods :

Test substance : thiram 80 WG (purity 81.06%)
Test species : Aphidius rhopalosiphi
Number of organisms : 5 males and 5 females X 4 replicates per treatment
Type of test : extended lab test
Applied and measured concentrations :
4 times at 1.92 kg a.s./ha (1-fold field rate), 2 times with 0.28 kg a.s./ha and 2 times with 0.15 kg a.s./ha (drift rate), water control and dimethoate control were also added to the test.
Exposure route :
Apple leaves were treated 4 times at interval of 7 days. Leaves were then used as floor of the arenas in which the adults were introduced
Test conditions :
Temperature : 20°C
Relative humidity : 50-85%
Continuous ligh (1000 lux) for exposure, 16 h light/8 h dark (2000-6000 lux) for reproduction

Findings :

Table B.9.5.2-2 : Effect of Thiram 80WG on *Aphidius rhopalosiphi* (extended lab test)

	Mortality* after 48 h [%]	Fecundity (mummies/female)
Water control	2.50	16.13
Toxic standard (dimethoate)	100	-
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	2.50	12.67
4 X 1.92 kg a.s./ha	5.00	13.47
	Corrected mortality* after 48 h [%]	Effect on reproduction [%]
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	0.00	21
4 X 1.92 kg a.s./ha	2.56	16

Conclusion :

No statistically significant effects neither in the mortality rates nor in the reproduction were observed in the two Thiram 80WG treated groups compared to the control group.

Thiram 80 WG: An extended Laboratory study to evaluate the effect on the ladybird *Coccinella septempunctata* L. (Coleoptera, Coccinellidae). (Stäbler, 2001)

Guidelines :

the guidelines of SCHMUCK *et al.* (2000)
the ESCORT I Guidance Document (BARRETT *et al.* 1994)
the ESCORT I Guidance Document and CANDOLFI *et al.* (2000)

GLP :

yes

Material and Methods :
Test substance : thiram 80 WG (purity 81.06%)
Test species : Coccinella septempunctata
Number of organisms : 50 larvae per treatment
Type of test : extended lab test
Applied and measured concentrations :
4 times at 1.92 kg a.s./ha (1-fold field rate), 2 times with 0.28 kg a.s./ha and 2 times with 0.15 kg a.s./ha (drift rate), water control and dimethoate control were also added to the test.
Exposure route :
Apple leaves were treated 4 times at interval of 7 days. Leaves were then used as floor of the arenas in which the larvae were introduced.
Test conditions :
Temperature : 22-27°C
Relative humidity : 55-74%
Light : 16 h light/8 h dark (2817-3370 lux)
Findings :

Table B.9.5.2-3 : Effect of Thiram 80WG on *Coccinella septempunctata* (extended lab test)

	Preimaginal Mortality [%]	Fecundity (number of fertile eggs/female/d)
Water control	2.00	8.12
Toxic standard (dimethoate)	100	-
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	18.00	7.06
4 X 1.92 kg a.s./ha	34.69	9.06
	Corrected preimaginal mortality [%]	
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	16.33	
4 X 1.92 kg a.s./ha	33.36	

Conclusion :
Fresh residue bioassay : harmless to *Coccinella septempunctata* for 4 applications at 1-fold field rate, harmless for 4 applications at the drift rate.

Thiram 80WG: An extended laboratory study to evaluate the effects on the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae). (Stäbler, 2001)
Guidelines :
- BIGLER (1988) and VOGT *et al.* (2000)
- ESCORT I (BARRETT *et al.* 1994) and ESCORT II (CANDOLFI *et al.* 2001)

GLP :
Yes
Material and Methods :
Test substance : thiram 80 WG (purity 81.06%)
Test species : Chrysoperla carnea
Number of organisms : 50 larvae per treatment
Type of test : extended lab test
Applied and measured concentrations :
4 times at 1.92 kg a.s./ha (1-fold field rate), 2 times with 0.28 kg a.s./ha and 2 times with 0.15 kg a.s./ha (drift rate), water control and dimethoate control were also added to the test.
Exposure route :

Apple leaves were treated 4 times at interval of 7 days. Leaves were then used as floor of the arenas in which the larvae were introduced.
2 bioassays were performed respectively with exposure to fresh residue and exposure to 28-day aged residue
Test conditions :
Temperature : 22-27°C
Relative humidity : 59-74%
Light : 16 h light/8 h dark (3165-3370 lux)

Findings :
Table B.9.5.2-4 : Effect of Thiram 80WG on *Chrysoperla carnea* – fresh residue bioassay (extended lab test)

	Preimaginal Mortality [%]	Fecundity (number of fertile eggs/female/d)
Water control	4.00	43.73
Toxic standard (dimethoate)	70.00	Not determined
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	28.00	25.73
4 X 1.92 kg a.s./ha	92.00	Not determined
	Corrected preimaginal mortality [%]	
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	25.00	
4 X 1.92 kg a.s./ha	91.67	

Table B.9.5.2-5 : Effect of Thiram 80WG on *Chrysoperla carnea* – 28-day aged residue bioassay (extended lab test)

	Preimaginal Mortality [%]	Fecundity (number of fertile eggs/female/d)
Water control	8.16	30.16
Toxic standard (dimethoate)	80.00	Not determined
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	10.00	31.01
4 X 1.92 kg a.s./ha	30.00	20.56
	Corrected preimaginal mortality [%]	
2 X 0.28 kg a.s./ha and 2 X 0.15 kg a.s./ha	2.00	
4 X 1.92 kg a.s./ha	23.78	

Conclusion :
Fresh residue bioassay : harmful effects to *Chrysoperla carnea* for 4 applications at 1-fold field rate, harmless for 4 applications at the drift rate.
28-day aged residue bioassay : harmless to *Chrysoperla carnea* for 4 applications at 1-fold field rate, harmless for 4 applications at the drift rate.

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

Foliar spray in orchards : The risk assessment for foliage dwelling arthropods has been completed .

Thiram is harmless to *Aphidius rhopalosiphi* (extended lab test available – 4 applications at 1-fold application rate)and to *Coccinella septempunctata* (extended lab test available – 4 applications at 1-fold application rate)

Thiram is harmful to *Chrysoperla carnea* (extended lab test available – 4 applications at 1-fold application rate)