THIRAM

Addendum to the monograph

September 2000

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

Table B.2.1-1 : Physical and chemical properties of thiram

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
B.2.1.12 Solubility in water : effect of pH (IIA 2.6)	- OECD-guideline 105 (flask method + HPLC-analysis) - No GLP-compliance stated	a.s. 99.8% pure : water solubility at 22 ± 2 °C : 17.9 mg/L (pH 4) 21.1 mg/L (pH 10) compared to water solubility at 20°C (neutral) : 16.5 mg/L Þ pH has no effect on solubility of thiram in water	- Not acceptable (study is not GLP + results appear to be incorrect)	Van de Putte, 1997a (UCB)
	- EEC-method A6 (flask method + HPLC-analysis) - GLP-compliance stated	a.s., 99.7% pure : solubility in distilled water at 10°C : 8.61 x 10^{-3} g/L (pH range 7.95 - 9.12) at 20°C : 1.46 x 10^{-2} g/L (pH range 7.25 - 8.98) at 30°C : 2.30 x 10^{-2} g/L (pH range 7.50 - 8.39) test was performed in distilled water only, as thiram is not capable of reversible protonation (determination of dissociation constant by spectroscopic method according to OECD-guideline 112 showed thiram to decompose under acidic and basic conditions)	- Acceptable	Flack, 1997 (FMC Foret)
B.2.1.14 Partition coefficient n-octanol/water : effect of pH (IIA 2.8)	- Applicant's statement	as pH has no effect on solubility of thiram in water, log P_{ow} is not influenced by pH	- Acceptable	
B.2.1.20	- EEC-method A10	technical a.s., 99.5% pure :	- Acceptable	Tremain, 1999 (Task

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
Flammability (IIA 2.11.1)	- GLP-compliance stated	no propagation of combustion in preliminary screening test Þ Thiram technical is not highly flammable		Force)
B.2.1.21 Auto-flammability (IIA 2.11.2)	- EEC-method A16 - no GLP-compliance stated	a.s., 99.8% pure : no sign of energy release between room temperature and melting process Þ Thiram is not auto-flammable	- Not acceptable (study is not GLP)	Van De Putte, 1997b (UCB)
	- EEC-method A16 - GLP-compliance stated	a.s., 99.7% pure : test substance did not self-ignite Þ Thiram is not auto-flammable	- Acceptable	Flack, 1997 (FMC Foret)
B.2.1.23 Explosive properties (IIA 2.13)	- EEC-method A14 - GLP compliance stated	technical a.s., 99.5% pure : · thermal sensitivity (effect of flame) : no explosion, no deformation of tubes · mechanical sensitivity (shock) : no traces of vigorous reaction · mechanical sensitivity (friction) : discoloration, no vigorous reaction ÞThiram technical is not explosive under the conditions of the test	- Acceptable	Krips, 2000 (Task Force)
	- EEC-method A14	a.s., 99.7% pure :	- The study is	Flack, 1997 (FMC

Study	Guidelines and GLP	Findings	Evaluation and conclusions	References
	- GLP-compliance stated	· thermal sensitivity (effect of flame) : no explosion, no deformation of tubes · mechanical sensitivity (shock) : with one test with 10 kg weight, the die was blown apart leaving a brown deposit (no audible reaction); all other 10 kg tests, as well as all of the 5 kg tests, were negative · mechanical sensitivity (friction) : no visible or audible reaction ÞThiram is not sensitive to heat or friction, but is slightly sensitive to shock ®Thiram has some explosive potential	acceptable. However, taking into account the results of the study by Krips, further clarification of the conclusion is required.	Foret)
B.2.1.24 Surface tension (IIA 2.14)	- EEC-method A5 - no GLP-compliance stated	a.s., 99.8% pure : σ = 56 mN/m (90% saturated aqueous solution)	- Not acceptable (study is not GLP)	Urbain, 1999 (UCB)
	- EEC-method A5 (OECD harmonised ring method) - GLP-compliance stated	a.s., 99.7% pure : σ = 72.7 mN/m at 18.5°C (90% saturated aqueous solution)	- Acceptable	Flack, 1997 (FMC Foret)

B.2.2 Physical and chemical properties of the plant protection products (Annex IIIA 2)

Table B.2.2-1 : Physical and chemical properties of **VITAVAX 200FF** (Flowable concentrate for seed treatment : thiram + carboxin) (UNIROYAL)

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.1 Appearance (IIIA 2.1)	- Applicant's statement	VITAVAX 200FF is a pink mobile flowable liquid with a slight characteristic odour	- Acceptable	Wiley, 1999a
B.2.2.4 Explosive properties (IIIA 2.2.1)	- EEC-method A14 - GLP-compliance stated	thermal sensitivity : negative mechanical sensitivity (shock) : negative Þ VITAVAX 200FF has no explosive properties	- Acceptable	Tremain & Bartlett, 1998
B.2.2.13 Surface tension (IIIA 2.5.3)	- EEC-method A5 (ring method) - GLP-compliance stated	1.02 g/L solution of VITAVAX 200FF : σ = 44.6 mN/m at 20.5°C Þ VITAVAX 200FF is considered to be surface active	- Acceptable	Hogg & Bartlett, 1998
B.2.2.17 Stability after storage	- Based on CIPAC MT 46	<u>after 12 weeks at 35°C in HDPE bottles</u> (3 samples tested):	- Acceptable	McKelvie, 1999

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
for other periods and temperatures (IIIA 2.7.1)	- GLP-compliance stated	1) carboxin content : 17.8% - 17.9% (initial : 17.7%) 2) thiram content : 17.4% - 17.5% (initial : 18.2%) ® max. decrease of 4.4% relative 3) no change in appearance (pink mobile flowable with no signs of sediment/claying and typical odour) 4) bleed on top surface : easily reconstituted (max. of 10 inverts) 5) wet sieve residue on 45 µ : 0.07% - 0.09% (initial : 0.07%) 6) pH (1% dilution) : 7.3 - 7.8 (initial : 7.1) 7) packaging : no signs of leakage or cracking ÞVITAVAX 200FF has good physical and chemical stability		
B.2.2.18 Minimum content after heat stability testing (IIIA 2.7.1)			- The information is not required since the content of thiram decreases with < 5 % relative.	
B.2.2.37		wheat and barley seed, treated with standard rates of	- Acceptable, as far as	Bartlett, 1999

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
Distribution and adhesion (IIIA 2.10)	- GLP-compliance stated	VITAVAX 200FF (applied either neat, co-applied with water or as a tank-mix with water) in Mini-Rotostat : <u>1) visual assessment of seed treatment quality (coverage, distribution, colour) :</u> tank-mix application with water is judged to be best treatment <u>2) chemical loading analysis :</u> - mean loading for carboxin and thiram : 88.5% (wheat) and 95.3% (barley) of the theoretical - co-application or tank-mixing with water gave higher chemical loadings (ca. 4%) over neat application - mean chemical losses (carboxin/thiram and wheat/barley) after storage under ambient conditions : < 3.7% (after 12 months) 4.2% - 8.7% (after 18 months) <u>3) adhesion tests (drop 10 times down a cilinder on to a sieve):</u> mean loss : 6.9% (wheat) and 3.5% (barley) at T0 7.6% (wheat) and 6.3% (barley) at T12 *Overall conclusions* : co-application or tank-mix of water with VITAVAX 200FF improved seed treatment quality in terms of both visual appearance and total chemical loading. The addition of water had however very little effect on storage or adhesion losses.	adhesion and seed loading is concerned. Distribution on the other hand was only assessed visually; determination of seed-to-seed uniformity of distribution according to CIPAC MT 175 was not included.	

Table B.2.2-2 : Physical and chemical properties of **THIRAM 80 WG** (water dispersible granule : thiram) (UCB)

Study	**Guidelines and GLP**	**Findings**	**Evaluation and conclusion**	**References**
B.2.2.4 Explosive properties (IIIA 2.2.1)	- Applicant's statement	The active substance, as well as the formulants, show no explosion sensitivity to shock, friction or elevated temperature. ÞTHIRAM 80 WG is expected to be non-explosive as well	- Acceptable (study by Krips (2000) on explosive properties of a.s. was considered to be acceptable)	
B.2.2.7 Flammability (IIIA 2.3)	- EEC-method A10 - GLP-compliance stated	preliminary screening test : combustion was propagated over the 200 mm in 507 seconds ÞTHIRAM 80 WG is not highly flammable	- Acceptable	Tremain, 1999
B.2.2.8 Auto-flammability (IIIA 2.3)	- EEC-method A16 - no GLP-compliance stated	no sign of energy release between room temperature and the melting process ÞTHIRAM 80 WG is not auto-flammable	- The study is not GLP, but taking into account the GLP-study on auto-flammability of the a.s. (see B.2.1.21), the results are nevertheless considered to be acceptable	Steyaert, 1999
B.2.2.10 pH of a 1 % aqueous dilution, emulsion or dispersion (IIIA 2.4.2)	- CIPAC MT 75.2 - GLP-compliance stated	1% aqueous dispersion of THIRAM 80 WG : pH = 8.35	- Acceptable	O'Connor & Mullee, 2000
B.2.2.15 Bulk or tap density	- CIPAC MT 159	tap density of THIRAM 80 WG (100 taps) = 0.633 g/ml (mean of 5 batches)	- Acceptable	Van Hecke, 1999a

Study (IIIA 2.6.2)	**Guidelines and GLP**	**Findings**	**Evaluation and conclusion**	**References**
B.2.2.21 Wettability (IIIA 2.8.1)	- CIPAC MT 53.3.1 (without swirling)	5 batches of THIRAM 80 WG : wetting time (range) : 5 - 10 seconds after 2 years storage at room temperature : wetting time (range) : 5 - 8 seconds	- Acceptable	Van Hecke, 1999b
B.2.2.26 Dry sieve test and wet sieve test (IIIA 2.8.5)	- CIPAC MT 167	5 batches of THIRAM 80 WG : wet sieve residue on 75 µm sieve (range) : 0.006 - 0.063% after 2 years storage at room temperature : wet sieve residue on 75 µm sieve (range) : 0.008 - 0.063%	- Acceptable	Van Hecke, 1999c
B.2.2.27 Size distribution of particles - Nominal size range of granules (IIIA 2.8.6.1)	- CIPAC MT 170 - GLP-compliance stated	particle size distribution range : x1 = 125 µm (R_{x1} ³ 90%) x2 = 425 µm (R_{x2} £ 10%)	- Acceptable	O'Connor & Mullee, 2000
B.2.2.28 Dust content and par-	- CIPAC MT 171 (gravimetric method)	collected dust = 0.9 mg	- Acceptable	O'Connor & Mullee, 2000

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
ticle size of dust (IIIA 2.8.6.2)	- GLP-compliance stated	Þ THIRAM 80 WG is “nearly dust-free”		
B.2.2.29 Friability and attrition characteristics of granules (IIIA 2.8.6.3)	- CIPAC MT 178 - GLP-compliance stated	attrition resistance : 95.9%	- Acceptable	O’Connor & Mullee, 2000
	- CIPAC MT 178	5 batches of THIRAM 80 WG : attrition resistance (mean) : 96.5% (range 91.4 - 98.8%)	- Acceptable	Van Hecke, 1999d

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B.3 Data on application and further information

B.3.5 Further information on the plant protection product VITAVAX 200FF - Seed treatment (Annex IIIA 4)

B.3.5.1 Packagings, suitability of the packaging material to its content (Annex IIIA 4.1)

VITAVAX 200FF is sold in 3 pack sizes within the EU (20 L, 200 L, 1000 L), as described below.

Tabel B.3.5.1-1 : Description of 20 L receptacle

Type :	blue pigmented HDPE container
Material :	High Molecular Weight High Density Polyethylene (HDPE)
Manner of construction :	extruded
Shape/size :	height : 39.0 cm depth : 24.5 cm width : 29.5 cm wall thickness : 1.4 mm (min.)
Weight :	1300 g (excluding closure)
Capacity :	nominal : 20 L maximum : 22.5 L
Opening :	63 mm
Closure/seal :	tamper evident, induction sealable with polyethylene
UN approval :	UN 3H1/Y 1.9/210/--

Tabel B.3.5.1-2 : Description of 200 L receptacle

Type :	cylindrical steel drum
Material :	steel with internal green phenolic lacquer
Manner of construction :	welded
Shape/size :	height : 880.3 mm diameter : 595.3 mm
Weight :	21.7 kg (nominal)
Capacity :	nominal : 200 L maximum : 216.5 L
Opening :	50 mm
Closure/seal :	lacquered steel with EPDM gasket
UN approval :	UN 1A1/X 1,6/280/--

Tabel B.3.5.1-3 : Description of 1000 L receptacle

Type :	natural HDPE container
Material :	High Molecular Weight High Density Polyethylene (HDPE)
Manner of construction :	extruded
Shape/size :	height : 1160 mm (including steel pallet) length : 1200 mm width : 1000 mm
Weight :	64 kg (including steel pallet)
Capacity :	nominal : 1000 L actual : 1060 L
Opening :	145 mm (top opening)
Closure/seal :	top closure : polyethylene plain screw cap and “o” ring seal lower discharge opening : protectively placed valve with outlet sealed by aluminium-lined polyethylene foil. Final closure by 50 mm polyethylene disc and “o” ring held within a screw cap
UN approval :	UN 31 HA1/Y/D/--

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B.4 Methods of analysis

B.4.1 Analytical methods for active substance and formulation analysis

B.4.1.1 Analytical methods for the determination of pure active substance in the active substance as manufactured (Annex IIA 4.1.1)

Uniroyal/UCB

- Control test AC-1099B : Determination of carboxin and thiram content in Vitavax technical, thiram technical and Vitavax/Thiram formulations by HPLC (Smilo, 1997)
- Thiram technical composition and identification of impurities : AC1099B Method validation - addendum to Project No. 93125 (Trudeau, 1996)

GLP :
No GLP-compliance stated
Principle of the method :
After dissolution of Thiram technical, resp. VITAVAX formulation, in internal standard solution (naphthalene in methanol), Thiram, carboxin and their impurities are separated by gradient HPLC (C18 column; 5 µm particles) and determined by UV detection at 254 nm.
Due to the instability of thiram in solution, it is important that the time for preparation and analysis be done within a 24 hr period.
Findings :
Specificity - interferences : the method resolves all known impurities and no interferences were found for Thiram technical material and carboxin/thiram formulations
Linearity : the response of the HPLC-UV system (= peak area) to thiram, resp. carboxin was found to be linear
: thiram : concentration range : 0.0102 - 0.51 g/L (n = 5)
r = 0.99786
carboxin : concentration range : 0.12 - 0.28 g/L (n = 5)
r = 0.99998
Accuracy : mean recovery determined using method of standard addition :
thiram technical : 101.04% (n = 6, RSD = 6.23%)
carboxin/thiram formulation : thiram : 99.09% (n = 5, RSD = 0.19%)
carboxin : 99.51% (n = 5, RSD = 0.63%)
Repeatability : thiram technical : RSD = 1.61% (n = 9)
carboxin/thiram formulation : thiram : RSD = 0.82% (n = 8)
carboxin : RSD = 0.80% (n = 8)
Conclusions :
The method is suitable for determination of thiram in thiram technical, as well as for determination of thiram and carboxin in thiram/carboxin formulations

FMC Foret

- Thiram (technical) : Five-batch analysis (Comb, 1997)

GLP :
GLP-compliance stated
Principle of the method :
Samples are dissolved in and diluted to volume with acetonitrile, after which the final solutions are analyzed by HPLC (Kromasil 100-5C18; 25 x 0.46 cm; mobile phase : water/methanol/acetonitrile (37:33:30)) with UV detection at 254 nm. Quantification by external standardization.
Findings :
Specificity - interferences : no interferences are mentioned
Linearity : the response of the HPLC-UV system (= peak area) to thiram was found to be linear
concentration range : 50 - 240 mg/L (n = 5)
y = 60.5 x + 27.1
r = 1.0000
Accuracy : demonstrated by linearity of assay system
sample prepared at 75% of nominal sample weight : 100.3% of reference value
sample prepared at 125% of nominal sample weight : 100.4% of reference value
Repeatability : RSD = 0.14% (n = 10)

Conclusions :
The HPLC-method is suitable for the determination of thiram in thiram technical.

B.4.1.2 Analytical methods for the determination of isomers, impurities and additives in the active substance as manufactured (Annex IIA 4.1.2)

Uniroyal/UCB

- Thiram technical composition and identification of impurities (Forbes, 1995)
- Accuracy validation of test control methods AC-1214 and AC-1216 for impurities in Thiram (Hull & Forbes, 1998)

GLP :
GLP-compliance stated
Principle of the methods :
1) impurity 1 (method AC-1215), impurity 2 (method AC-1214) and impurity 3 (method AC-1216): Thiram and its impurities are separated by gradient HPLC (column C18, 5 µm particle, 220 X 4.6 mm (I.D.), gradient programming with water/methanol eluents) and quantitatively measured by UV detection (254 nm) using naphtalene as internal standard. (for impurity 1 : identification by MS)
2) impurity 4 : determination by drying of the product in an oven at 100°C
3) impurity 5 (GRL-SOP-1046) : samples are mineralized in a muffle furnace at 750-850°C until constant weight is obtained.
Findings :
see Table B.4.1.2-1

Table B.4.1.2-1 : Validation of methods for determination of impurities in technical thiram - Uniroyal

Impurity	Specificity/interferences	Repeatability (% RSD)	Mean recovery (%)	Linearity
0,04167	no interferences are mentioned	4.5 (n = 20)	-	n = 4 (0.1 to 1.0 mg/ml) r = 0.9998
1b		4.2 (n = 20)		quantification using thiram as surrogate standard (not possible to synthesize impurity 1 for use as reference standards due to their instability)
2	no interferences are mentioned	2.5 (n = 20)	99.65 (n = 4; RSD = 0.62%)	n = 4 (0.004 to 0.1 mg/ml) r = 0.9999
3	no interferences are mentioned	2.9 (n = 8)	101.04 (n = 4; RSD = 1.64%)	n = 4 (0.004 to 0.03 mg/ml) r = 0.9996
4	generally accepted method			
5	generally accepted method			

Conclusions :
Suitable methods are available for determination of significant impurities in thiram technical.

UCB

- Composition of Thiram Phyto, Thiram 80 WG, Ziram Phyto, Ziram 76 WG (Van De Putte, 1995)

GLP :
No GLP-compliance stated

Principle of the methods :
1) additive 6 : a sample of technical thiram is extracted 3 times with 20 mL of water, followed by filtration on a glass fiber filter. The collected filtrate is diluted with water and subjected to UV spectroscopic analysis at 280 nm. Quantification by external standard calibration.
2) additive 7 : an analysis method to determine small quantities of additive 7 in pesticides could not be developed until now
Findings :
no validation data
Conclusions :
No validated methods are available for determination of additives in thiram technical.
UCB states that it is not relevant to develop further methods for this, as the additives are not of any toxicological concern and the quantities in which they are added are known.

FMC Foret

- Thiram (technical) : Five-batch analysis (Comb, 1997)
GLP :
GLP-compliance stated
Principle of the method :
1) impurity 1 and 2 : samples are dissolved in and diluted to volume with acetonitrile, after which the final solutions are analyzed by HPLC (Kromasil 100-5C18; 25 x 0.46 cm; mobile phase : water/methanol/acetonitrile (37:33:30)) with UV detection at 254 nm. Quantification by external standardization.
2) impurity 3 : Karl Fischer titration method
Findings :
see Table B.4.1.2-2

Table B.4.1.2-2 : Validation of methods for determination of impurities in technical thiram - FMC Foret

Impurity	Specificity/interferences	Repeatability (% RSD)	Mean recovery (%)	Linearity
1	no interferences are mentioned	4.18 (n = 5)	127 (0.3% w/w level; n = 3) 119 (0.6% w/w level; n = 3)	n = 8 (0.01 - 2.2 mg/L) y = 24.0 x - 0.332 r = 0.9995 LOD = 0.001 mg/L
2	no interferences are mentioned	17.5%* (n = 5)	98.5 (0.3% w/w level; n = 3) 99.0 (0.6% w/w level; n = 3)	n = 8 (0.01 - 2.2 mg/L) y = 123 x - 1.25 r = 0.9999 LOD = 0.01 mg/L
3	generally accepted method			

* concentration level in samples was less than 0.01%

Conclusions :
Suitable methods are available for determination of significant impurities in thiram technical.

B.4.1.3 Analytical methods for the determination of pure active substance in plant protection products (Annex IIIA 5.1.1)

see B.4.1.1

B.4.2 Analytical methods (residue) for food and feed (Annex IIA 4.2.1; Annex IIIA 5.2.1)

We currently await evaluation of new information regarding the presence of metabolite M1 in the metabolism of the rat.
If the provisional residue definition (thiram) is confirmed, the specific HPLC-method that was included in the monograph is acceptable and no further methods will be required. Otherwise, a specific method could be requested to determine M1.

B.4.3 Analytical methods (residue) in soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2.2 to 5.2.4)

B.4.3.1 Analytical methods for soil and water (Annex IIA 4.2.2 and 4.2.3; Annex IIIA 5.2.2 and 5.2.3)

An analytical method for determination of thiram residues in soil is under development and will be submitted by 31/12/00

A specific method for determination of thiram residues in water is available and will be submitted.

B.4.3.2 Analytical methods for air (Annex IIA 4.2.4; Annex IIIA 5.2.4)

- Method 5005, issue 2 (NIOSH Manual of Analytical Methods, Fourth edition - 15/8/94)
Principle of the method :
Thiram residues in air are trapped on a 1 µm PTFE membrane filter, held in a two-piece polystyrene cassette filter holder with backup pad, using air sampling at rates of 1 to 4 L/min for a total sample size of 10 to 400 L (total dust loading on filter should not exceed 2 mg).
The filter is placed in a 20 mL vial, 10 mL acetonitrile is added and the vial capped, after which the samples are agitated during a 30 min desorption period.The inside top of the cassette is rinsed with 10 mL of acetonitrile into another 20 mL vial. Residues are determined by HPLC (Bondapak C18; 300 x 3.9 mm) with UV detection at 254 nm. Quantification by external standard calibration.
Findings :
Specificity - interferences : no interferences are known
Accuracy : average recovery = 99.8% (n = 3x6; pooled S.D. = 0.012)
Repeatability : pooled S.D. = 0.022 (n = 3x6, samples collected from test atmospheres)
Sample stability : 7 days at 25°C
Working range : 0.1 to 3 mg per sample (corresponds with 0.5 to 15 mg/m3 for a 200 L air sample)
LOD : estimated at 0.005 mg per sample (corresponds with 0.025 mg/m3 for a 200 L air sample)
Conclusions :
Method 5005, issue 2 is a validated, officially accepted method (USA)

B.4.4 Analytical methods (residue) wildlife and for use in support of diagnostic and therapeutic regimes (Annex IIA 4.2.5; Annex IIIA 5.2.5)

An analytical method should be developed : long term risk was identified for birds eating seeds.

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B.7 Environmental fate and behaviour

B.7.3 Summary of behaviour in soil and predicted environmental concentration in soil (PECs) Annex IIIA 9.1.3)

The rate of degradation under aerobic conditions was determined in 3 soils, at 20°C and in 1 soil at 10°C.

Table B.7.3-1 : Summary of behaviour in soil (new study)

Test soil				**Test system**	**Results**	**References**
Soil type	**Soil $pH_{(KCl)}$**	**CEC**	**Organic carbon - content**			
sandy loam	6.1	5.62	1.05	Aerobic degradation at 20° C	DT_{50} = 3.73 days DT_{90} = 12.4 days	Nag et al., 2000
sandy clay loam	6.9	3.8	24.9		DT_{50} = 5.51 days DT_{90} = 18.3 days	
clay	7.8	39.9	39.9		DT_{50} = 6.82 days DT_{90} = 61.3 days	
sandy loam	6.1	5.62	5.62	Aerobic degradation at 10° C	DT_{50} = 9.83 days DT_{90} = 32.7 days	
sandy loam	6.1	5.62	1.05	Aerobic degradation at 20° C	DT_{50} (DMCS) = 15.0 days DT_{90} (DMCS) = 49.8 days	Nag et al., 2000
sandy clay loam	6.9	3.8	24.9		DT_{50} (DMCS) = 37.6 days DT_{90} (DMCS) = 124.8 days	
clay	7.8	39.9	39.9		Data too variable	
sandy loam	6.1	5.62	5.62	Aerobic degradation at 10° C	DT_{50} (DMCS) = 38.7 days DT_{90} (DMCS) = 128.7 days	

DMCS : N,N-dimethylcarbosulfonate

PEC soil :
PEC were calculated for the seed treatment applications and for the foliar applications in orchard.

The following assumptions were made to calculate the PEC for seed treatment applications :
- DT_{50} of 6.4 days was taken as worst case. The degradation was calculated according to first order kinetics.
- First order dissipation PEC and TWA PEC were calculated for the 'worst case' situation : peas at the highest sowing rate.
- Several major crops were taken into account.
- soil layer of 5 cm and soil density of 1.5 g/cm3
- sowing rates according to the ATD Guide to the International Seed Business, Agritech Decision, (1991) and to Station of Phytopharmacy , Gembloux
-' Worst cases' were the application rates of formulations containing thiram only.

Table B.7.3-2 : PEC soil of thiram in mg a.s./kg soil - seed treatment

Crop	**Sowing rate (kg seed/ha)**	**Application rate (g a.s./100 kg seed)**	**Initial PECsoil (mg a.s./kg soil)**
Barley	120-155	200	240-310
Oat	120-130	150	180-195
Rye	115-150	200	230-300
Wheat	170-200	160	272-320
Pea	100-220	200	200-440
Maize	20-25	200	40-50
Sugar beet	40-50	480	192-240
Oilseed rape	2-5	440	8.8-22

Table B.7.3-3 : PEC soil of thiram in mg a.s./kg soil - Seed treatment

Time after application (days)	Sowing of peas 220 kg seed , 200 g a.s./100 kg seed	
	first order dissipation PEC	TWA PEC
0	0.587	0.587
1	0.526	0.556
2	0.472	0.527
4	0.380	0.476
7	0.275	0.411
14	0.129	0.302
28	0.028	0.184
50	0.003	0.108
100	0.000	0.054

The following assumptions were made to calculate the PEC for foliar applications :

- DT_{50} of 6.4 days was taken as worst case. The degradation was calculated according to first order kinetics.
- TWA PECs were calculated according to the recommendations of the FOCUS group (Doc 7617/VI/96 - Feb. 97)
- 50 % of the a.s. reached the soil.
- soil layer of 5 cm and soil density of 1.5 g/cm3
- Two scenarios were chosen :
  grapes, 3.2 kg a.s./ha, 3 applications/season, 7 days interval
  orchard, 2.4 kg a.s./ha, 4 applications/season, 7 days interval

Table B.7.3-4 : PEC soil of thiram in mg a.s./kg soil - foliar application

Time after application (days)	Grapes 3.2 kg a.s./ha 50% a.s. reaching the soil		Orchard 2.4 kg a.s./ha 50% a.s. reaching the soil	
	actual residue (mg/kg)	TWA (mg/kg)	actual residue (mg/kg)	TWA (mg/kg)
0	2.133	2.133	1.600	1.600
1	1.914	2.022	1.436	1.516
2	1.718	1.918	1.288	1.439
4	1.383	1.731	1.037	1.268
7	3.129	3.131	2.350	2.350
14	3.597	3.599	2.701	2.701
21	1.687	2.525	2.865	2.865
28	0.791	1.854	1.342	2.009
42	0.370	1.421	0.295	1.130
50	0.073	0.905	0.124	0.873
100	0.000	0.387	0.001	0.335

B.7.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2)

B.7.4.4 Water/sediment study (Annex IIA 7.2.1.3.2)

First amendment to report Degradation and metabolism of thiram in aquatic systems (Wyss-Benz M., 1992) (Mamouni, 2000)
Conclusions :
Two known metabolites (dimethyldithiocarbamic acid methyl ester and CS_2) were detected in the water phase; Several unknown metabolites were detected at a rate higher than 10% during the incubation period. Identification of these metabolites of concern was not performed.

Degradation and metabolism of thiram in an aquatic system (Wyss-Benz M., 1995)
This report describes the additional investigation on the degradation and the metabolism of the fungicide ^{14}C-THIRAM, i.e. tetramethyl[^{14}C]-thiuram disulphide, in an aerobic aquatic system originated from the river Rhine. A normal water/sediment study was already performed under RCC project number 303456 with the maximum recommended field rate of 3.2 kg a.i./ha or 1.1 mg a.i./l water. In the present study, the application rate was much lower, i.e. 16 µg a.i./l, 10 times the calculated PEC (predicted environmental concentration) of 1.6. µg/l.

The incubation period at 20°C ± 2°C in the dark was 10 days with the sampling intervals 0, 0.25, 0.75, 2, 7 and 10 days. A volume of 530 ml of water and 300 g of sediment (wet) were filled into 1 liter metabolism flasks. The system was aerated and the oxygen concentration, pH and redox potential of the water was measured until an equilibration in the system was achieved after about 27 days.

The main degradation product was volatile, i.e. CO_2, which accumulated to 21.0 % of the radioactivity applied within the study. Other volatile substances were 4.7 % on day 10, assumed to be mainly CS_2 because of the results of the former study.

In this former study (RCC project number 303456), the very polar radioactive fractions were detected by HPLC at the pass through or with a shortly higher retention time. In the present study, the very polar fractions remained in the water-phase after partition and were analysed by TLC. They corresponded with each other in both studies.

Nine different unknown radioactive fractions MW1 to MW9 were observed. MW2 was the main fraction which increased to 11,7 % of the radioactivity applied or to 1.9 µg p.e./l on day 10. MW1, MW3, MW5, to MW8 reached a maximum after 2 days of incubation, i.e. MW1 7.4 %; MW3 8.0 %; MW5 2.0 %; MW6 6.6 %; MW7 2.3 %; MW8 0.8%; and all decreased or disappeared until day 10. MW4 showed its maximum on day 0.75 with 7.0 % and decreased thereafter, too. MW9 was highest on day 0.25 with 5.5 % and disappeared on day 7. DMDTC-ME was the only reference compound which was detected on days 7 and 10 with 1.2 % and 0.8 %, respectively. Another unknown, less polar radioactive fraction, i.e. U at a level of 1.2 %, was only detected on day 2 in the organic partition by HPLC.

It can be concluded that under aerobic conditions THIRAM is very fast degraded to several polar substances and is then mineralised.

THIRAM

Addendum to the monograph

September 2000

B.8 Ecotoxicology

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

Toxicity of Vitavax 2000 to game animals under field conditions (Zajak, 1990)
GLP :
no
Material and Methods :
Test substance: VITAVAX 200FF (containing 170 g/l carboxin and 170 g/l thiram)
Test species : Common pheasant *(Phasianus colchicus)*
Experimental design :
The objective of this field experiment was to assess the effects of Vitavax 200 coated maize seeds on the behavioural and feeding patterns as well as the reproductive performance of pheasants occurring in the surroundings of the treated maize field during the breeding season.
2 locations in Hungary were selected. One field treated with Vitavax (maize, 360 ppm thiram + 360 ppm carboxin) and one field treated with Buvisild K (maize, 900 ppm captan) as control were sown in both locations. Treated fields were 205 and 328 ha large.
Findings :
*Mortality :*during the period of sowing and sprouting of maize the pheasant populations showed no decreases in number.
Behaviour : the birds exhibit no changes in behaviour and responding ability.
Signs of sickness and dead animals were not noted.
Food consumption : Maize seed consumption indicated only a slight repelling effets
Gross findings :
In one out of 6 hens shot lesions similar to those induced by thiram were noted : in the uteri the egg was much greater in size than normal, irregular shaped.
Histopathological findings : Histopathological examinations revealed acute serous imflammation of the uteri in 4 of 6 pheasants shot in the experimental field. In one hen with a positive gross findings the lesion was accompanied with a marked formation of fibrine coating both the mucosal and serosal surface of the uterus.
Pheasant progeny : No considerable differences between treated and control fields were found in the proportion of hens with chickens.
The first young chicks appeared in the treated fields 5-10 days later than in other sites, probably indicating the interference of Vitavax with the egg formation which resulted a delay in brooding and hatching.
Conclusions :
In this experiment which was realized under conditions similar to the agricultural practice (large fields, normal sowing technique, wild animals, other food sources available) an unacceptable impact on the reproduction was observed.

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

Repulsive efficiency of thiram, used as seed treatment, for the red-legged partridge (Alectoris rufa) (Grolleau, 2000)
Guidelines :
In-house protocols
GLP :
no
Material and Methods :
Test substance: FLOWSAN FF (containing 533 g/l thiram), anthraquinone
Test species : red legged partridge (*Alectoris rufa*)
Sex, weight, age : subadults partridges, 5 males and 5 females per factor
Experimental design :
Groups of 10 partrigdes were offered treated maize with anthraquinone or thiram in order to measure their reppellent effects. Partridges weights and food intakes were measured
Findings :

	thiram	anthraquinone	
decrease in intake without food choice	84.2%	45.1%	UCB-INRA protocol

decrease in intake without food choice	51.4%	23.3%	UCB protocol
% total intake with food choice	5.7%	11.9%	UCB protocol

Conclusions :
Both protocols lead to the conclusion that 1600 ppm of thiram on maize seeds is more repellent than 1000 ppm anthraquinone. This experiment showed that some intake occurs despite the repellent effect of thiram.

Repellent effect of thiram on partridges under laboratory conditions (Grolleau, 1998)
Experimental design :
After acclimatisation in individual cages, 12 partridges/batch were fed with neutral wheat for 3 days. The design of the cages allowed to weigh accurately the daily consumption of each bird. On day 4, the birds were fed with treated seeds in order to evaluate the 'instantaneous' repellent effect. (consumption drop = consumption of treated seeds/ mean consumption of neutral seeds)
Findings :

		Grey partridge (*Perdrix perdrix*)	Red-legged partridge (*Alectoris rufa*)
Anthraquinone	1600 ppm 1600 ppm 3200 ppm 6400 ppm	-29.9% -28.5% -55.9% -82.7%	-41.0% -32.7% -73.6% -91.2%
Thiram	1000 ppm 2000 ppm 4500 ppm	-66.3% -69.9% -91.8%	-56.5% -66.0% -91.8%

Conclusions
Differences in efficacy between 1000 and 2000 ppm are low. If sub-total efficacy is sought, the level must be increased to 4500 ppm in the absence of choice of feed.

Tests in the open (in nature) of the repellency of Vitavax 200 towards the rook (*Corvus frugilegus*)
Experimental design :
Tests made according to the protocol CEB n° 118, taking anthraquinone as reference.
Tests were installed near rookeries in 2 locations in February-March 1992.
During the period of familiarization (at least 2 weeks) , untreated maize in piles of 2 kg each is placed in the same way and on the same plots as those which will be used in the test.
During the test, 2-kg piles of maize are offered (4 piles for each treatment). At the end of each week maize remains were weighed. To avoid the effect of position of blocks with the flightpath of the birds, treatments were switched from week to week.

Findings :
Consumption of treated maize in the open - test n°1

	anthraquinone 560 ppm	Thiram 500 ppm	Thiram 1000 ppm

Week 1	14.9%	0.5%	0.2%
Week 2	20.1%	1.1%	1.2%
Week 3	50.5%	2.6%	4.3%

Consumption of treated maize in the open - test n°2

	anthraquinone 560 ppm	Thiram 500 ppm	Thiram 1000 ppm
Week 1	31.0%	5.9%	6.0%
Week 2	50.9%	22.4%	16.0%
Week 3	53.8%	44.5%	26.3%

Conclusions :
This test design is very close to 'real world'. In these tests thiram was more repellent than anthraquinone. Maize treated at 1000 ppm showed a better repellency effect than maize treated at 500 ppm. After 2 weeks rooks partially overcome the repellency.
On the efficacy point of view, thiram should be used preferably at the dose of 1000 ppm as bird repellent.
On the ecotoxicology point of view, this study shows that some consumption occurs. Exposure of the birds to thiram is therefore possible.

Thiram Technical: A palatablity/repellancy study with the Northern bobwhite (*Colinus virginianus*) (Beavers, 1995)
GLP test performed according to the FIFRA guideline.
Northern bobwhite received either diet containing thiram technical at 10, 100, 500 or 2500 ppm, or a choice between basal diet and diet containing thiram technical at 10, 100, 500 or 2500 ppm for 14 days.
Two pens of five birds were evaluated in each group. A control group receiving basal diet was maintained concurrently. The exposure period was followed by a seven day observation period during which all birds received basal diet. During the test, the birds were observed daily for mortality, abnormal behaviour and signs of toxicity. In addition body weight and feed consumption were measured.
Findings :
Feed consumption results - Northern bobwhite

Nominal concentration	Week 1	Week 2	Week 3 (observation period - basal diet only)
males			
0 ppm	59/41	60/40	52/48
basal/10 ppm	47/53	44/56	34/66
basal/ 100 ppm	39/61	50/50	45/55
basal /500 ppm	69/31	70/30	39/61
basal /2500 ppm	86/14	87/13	57/43
females			
0 ppm	61/39	60/40	56/44
basal/10 ppm	57/43	56/44	61/39
basal/ 100 ppm	62/38	41/56	58/42
basal /500 ppm	57/43	54/46	51/49

basal /2500 ppm	92/8	87/13	66/34

Consumption of groups without choice were not indicated in this table.
Conclusions :
Some repellency is observed at 2500 ppm in females, at 500 and 2500 ppm in males.
No mortality, no overt signs of toxicity or treatment related body weight effects were observed at any dose (groups with basal/treated ration, groups with treated ration). However some slight signs of toxicity were observed at 2500 ppm (no food choice)

Thiram Technical: A palatablity/repellancy study with the mallard (*Anas platyrhynchos*) under multiple choice conditions (Beavers, 1995)
GLP test performed according to the FIFRA guideline.
Mallard received either diet containing thiram technical at 10, 100, 500 or 2500 ppm, or a choice between basal diet and diet containing thiram technical at 10, 100, 500 or 2500 ppm for 14 days.
Two pens of five birds were evaluated in each group. A control group receiving basal diet was maintained concurrently. The exposure period was followed by a seven day observation period during which all birds received basal diet. During the test, the birds were observed daily for mortality, abnormal behaviour and signs of toxicity. In addition body weight and feed consumption were measured.
Findings :
Feed consumption results - Mallard

Nominal concentration	Week 1	Week 2	Week 3 (observation period - basal diet only)
males			
0 ppm	51/49	50/50	62/68
basal/10 ppm	74/26	74/26	77/23
basal/ 100 ppm	70/30	70/30	48/52
basal /500 ppm	86/14	90/10	76/24
basal /2500 ppm	75/25	93/7	46/54
females			
0 ppm	51/49	28/72	20/80
basal/10 ppm	61/39	70/30	49/51
basal/ 100 ppm	65/35	67/32	48/52
basal /500 ppm	92/8	90/10	65/35
basal /2500 ppm	95/5	96/4	28/72

Consumption of groups without choice were not indicated in this table.
Conclusions :
Among birds receiveing a choice between treated diet and basal diet, feed consumption measurements indicated a concentration reponsive preference for basal diet during the exposure period.
No mortality, no overt signs of toxicity or treatment related body weight effects were observed in the groups with basal/treated ration.
Signs of toxicity were observed in the groups with treated ration (500 and 2500 ppm without food choice)
However some slight signs of toxicity were observed at 2500 ppm (no food choice)

One palatability study performed with formulation VITAVAX 200FF (thiram + carboxin) showed repellency effects on pheasant. (Described in the original monograph)

Acceptance/repellence test of maize seeds treated with Flowsan FS in pheasant (Phasianus colchicus) (Szakolczi, 2000)
GLP test performed according to the BBA guideline.
Maize seeds were treated with Flowsan FS (533 g a.s./l) at the rate of 1275 mg a.s./kg seeds. Untreated seeds (75%; maize, wheat, sunflower) and treated seeds (25%; maize, red-colored) were dispersed on the feeding area at the same time, 3 times for 8-hour period. Every exposure was preceded by 16-hour fasting.
5 male and 5 female pheasants (Phasianus colchicus) did not consume the treated seeds. During the test period neither clinical symptoms nor any damage to the birds' health were observed. No mortality occurred.

Vitavax 200 FF - Assessment to determine the effect on palatability and dietary toxicity in the pigeon (Johnson, 1999)
GLP test performed according to the BBA guideline (Accelerated acceptance test). Untreated seed and treated seed are scattered on the floor . Observation of the birds pecking on the floor (1 hour duration, for 3 days) . No measurement of food consumption.
This test seems very inconclusive.

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

All the information on birds which was submitted after completion of the monograph was summarized here.

Summary of effects of thiram to birds

Test species	**Test system**	**Duration of exposure**	**Results**	**References**
Mallard	Acute oral test	single exposure	LD50 > 2000 mg/kg	Zajak, 1995
Japanese quail	Acute oral test	single exposure	LD50 > 2000 mg/kg	Zajak, 1995
Mallard	Short-term dietary	5 days	LC50 > 5247 mg/kg food	Zajak, 1995
Japanese quail	Short-term dietary	5 days	LC50 > 4423 mg/kg food	Zajak, 1995
Bobwhite quail	Reproduction	23 weeks	NOEC = 500 mg a.s./kg food 2/12 cases of ovary regressing were observed at 500 and 2500 mg/kg food Severe effects on reproduction parameters were observed at 2500 mg/kg food	Beavers, 1995

Acute and dietary toxicity studies were performed with Biosild T, a formulation containing 492 g/l thiram)

Literature data on the toxicity of thiram (mentionned in the monograph)

Test species	**Test system**	**Duration of exposure**	**Results**	**References**
Japanese quail	Acute oral test	single exposure	LD50 = 695 mg a.s./kg	Gruen et al., 1982
Bobwhite quail	Short-term dietary	5 days	LC50 = 3950 mg a.s./kg	Hill et al. 1975

Information from the open literature was briefly summarized. These studies confirm the effects of thiram and ziram on bird reproduction
125 ppm thiram in the diet of laying hens stopped egg production within 2 daysand caused marked ovarian atrophy after 7 days (Weppelman, 1980)
50 ppm thiram in the diet of Japanese quails caused reduction of egg production and had incidence on eggshells (Egberts , 1972)

At concentrations > 25 ppm, thiram had effects on the reproduction of Japanese quails (Riedel, 1986)
100 ppm thiram in the diet of red partridges caused reduction of egg laying and reduction of hatching (Grolleau 1966)
Thiram caused weight loss and effects on the nervous system and male reproduction system of fowls (Rasul, 1974)
40 ppm thiram in the diet of chicks produced toxic effects; turkeys would be less sensitive (Waibel, 1955, 1957)
Thiram greatly affects the birds general conditions and totally inhibited egglaying of the red partridges (Soyez, 1978)
Thiram inhibits ovualtion and reduced egg laying in the bobwhite quails (Wedig, 1968)

Egberts J., Jannekerdoos and Beijer J.M.	The toxicity of TMTD in Japanese quail, an experimental study	TNO - nieuws 594-598 (1972)
Grolleau G. and Biaddi F.	Note on the effects of thiram on the laying and rearing of the red-legged partridge (alectoris rufa)	J. Appl. Ecol. (suppl.), 3, 249-251, (1966)
Hornshaw t. C. Aulerich R.J. and Ringer R. K.	Toxicity of Thiram (Tetramethylthiuram Disulfide) to Mink and European Ferrets	Bull. Environ. Contam. Toxicol. (1987) 38, 18-62, (1987)
Rasul A.R. and McC Howell J.	The Toxicity of Some Dithiocarbamate Compounds in Young and Adult Domestic Fowl	Toxicology and applied Pharmacology 30, 63-78, (1974)
Riedel Bernd und Grün Gerhard	Die vogeltoxikologische Bewertung von Thiram, Carboxin und Carbendazim als Saatgutbeizmittel für Getreide	Nachrichtenblatt Pflanzenschutz DDR, 40, 147-151, (1986)
Soyez D., Lorgue G.	Etude et comparaison des effets toxiques du thirame, du zirame, du zinebe, du manèbe et du mancozèbe Fongicides du groupe des dithiocarbamates sur les perdrix rouges	Bull. mens. Off. Nation. Chasse, 58-66, (1978)
Waibel P. E.,. Johnson Elton L, B. S. Pomeroy and Howard L. B.	Toxicity of Tetramethylthiuram Disulfide for Chicks, Poults, and Goslings	Poultry Science, 36, 697-703, (1957)
Waibel P.E., Pomeroy B.S., Johnson Elton L.	Effect of Arasan-Treated Corn on Laying Hens	Science 121, 401-402, (1955)
Wedig J., A. Cowan, and Hartung R.	Some of the Effects of Tetramethylthiuram Disulfide (TMTD) on Reproduction of the Bobwhite Quail (Colinus virginianus)	Toxicol. Appl. Pharmacol, 12, 293, (1968)
Weppelman R.M., Long A.A., Van Iderstine A., Taylor J.E., Tolman R., Peterson L. and Olson G.	Antifertility ffects of Dithiocarbamates in Laying Hens	Biology of reproduction 23, 40-46, (1980)

The evaluation of the risk in the case of seed treatment

The risk assessment is based on the following assumptions :
- The application rate on maize or pea was chosen (200 g a.s./100 kg seed or 2000 mg a.s./kg food)
- The ‘nominal’ rate of application was taken into account assuming that no degradation of the a.s. occurred just after sowing.
- Scenarios for small birds (30% food consumption) were considered.

Conclusions :
The TER acute and short term are slightly below the trigger value of 10. However this evaluation may be considered as a worst case situation since it does not take into account the possible degradation of thiram in the soil or at its surface.

The calculation of the TER long term reveal an unacceptable high risk. Results of the field experiment (Zajak, 1990), the reproduction toxicity study (Beavers, 1995), and numerous references of the open literature confirm these conclusions. Thiram has detrimental effects on the male and female reproduction system of birds even after a reduced intake of treated seeds during a short exposure period . This leads to reduced egg production, delay in hatching,....
There is no safe use of thiram as seed treatment.

Several repellency studies have shown that consumption of treated seeds can't be completely excluded. Repellent effect would not be sufficient to avoid a limited consumption of treated seeds which would be detrimental.

Toxicity exposure ratios (TER's) for birds exposed to thiram - Seed treatment 2000 ppm

Bird type	**food consumed**	**Effect concentration**	**Estimated Theorical Exposure**	**Type of TER**	**Toxicity Exposure Ratio**
small bird (<100g bw)	cereals, pea or maize	> 2000 mg a.s./kg bw	600 mg a.s./kg bw	LD_{50}/ETE	3.31
		> 4423 mg a.s./kg feed	2000 mg a.s./kg feed	LC_{50}/ETE	2.21
		500 mg a.s./kg feed	2000 mg a.s./kg feed	NOEL/ETE	0.25

Toxicity exposure ratios (TER's) for birds exposed to thiram - Seed treatment - new GAP proposal of Uniroyal 600 ppm

Bird type	**food consumed**	**Effect concentration**	**Estimated Theorical Exposure**	**Type of TER**	**Toxicity Exposure Ratio**
small bird (<100g bw)	cereals	> 2000 mg a.s./kg bw	180 mg a.s./kg bw	LD_{50}/ETE	11.11
		> 4423 mg a.s./kg feed	600 mg a.s./kg feed	LC_{50}/ETE	7.37
		500 mg a.s./kg feed	600 mg a.s./kg feed	NOEL/ETE	0.83

The evaluation of the risk in the case of foliar spray in orchard and vineyard

The risk assessment is based on the following assumptions :
- The application rate of 3.2 kg a.s./ha in vineyard was taken as 'worst case' situation.
- The initial concentration of thiram in the food was estimated according to Hoerger and Kenaga (1972)
- Scenarios for small birds (30% food consumption) and large birds (10% food consumption) were evaluated.

The evaluation of the different TER (44.6- 67.2) showed that the acute and short term risk resulting from the use of thiram in vineyard and orchard is low. The long term risk is borderline (TER = 5) . Due to the important reproductive effects which were observed in several studies, this risk evaluation should be refined.

Estimated initial concentration of thiram in potential feed of birds - Foliar application

Target crop	**Application rate**	**Estimated initial residues (mg a.s. kg food)**

	(g a.s./ha)			
		Leaves and leafy crops	**Small seeds and small insects**	**Large insects**
Vineyard	3200 g a.s./ha	99.2	92.8	8.64

Toxicity exposure ratios (TER's) for birds exposed to thiram - Foliar applications - worst cases

Bird type	**food consumed**	**Effect concentration**	**Estimated Theorical Exposure**	**Type of TER**	**Toxicity Exposure Ratio**
small bird	small seeds and small insects	> 2000 mg a.s./kg bw	29.76mg a.s./kg bw	LD_{50}/ETE	67.2
		> 4423 mg a.s./kg feed	99.2 mg a.s./kg feed	LC_{50}/ETE	44.6
		500 mg a.s./kg feed	99.2 mg a.s./kg feed	NOEL/ETE	5

Summary of effects to mammals - exposure and risk assessment for mammals (Annex IIIA 10.3)

The antifertility and antiadrenergic actions of thiocarbamate fungicides in laying hens. (Serio et al., 1984)
Hypothesis of the authors
The antifertility activity of thiram and ziram would be related to their inhibiting effect on the dopamine □-hydroxylase (enzyme catalyzing the transformation of dopamine to norepinephrin in the brain)
Norepinephrin is involved in a chain of hormones regulating the function of reproduction :

Norepinephrin => LHRH (hypothalamus) => LH, FSH (pituary) => oestrogen, progesterone (ovaries)
testosterone (testes)

Findings :
1) The first part of the study (in vivo + in vitro) showed that norepinephrin synthesis is inhibited from 2.5 mg a.s./kg bw/d onwards for thiram. => NOAEL = 1 mg a.s./kg bw/d

The study showed that norepinephrin synthesis is inhibited from 2.5 mg a.s./kg bw/d onwards for ziram. => NOAEL = 1 mg a.s./kg bw/d

2) The second part of the study (in vivo) showed that thiram when fed to laying hens for 1 week caused
- at 250 ppm : ovarian weight regression
- at 125 ppm (6.25 mg/kg bw/d): nonsignificant ovarian weight reduction. These results suggest that the NOAEL is slightly < 6.25 mg/kg bw/d
However the concentrations of norepinephrin in the brain and in the heart were not related to treatment. The results of the study do not fit completely with the hypothesis of the authors

Several toxicological studies (presented in the monograph) described the effects of thiram on the reproduction.

- 90 d rat study (Mishra et al. 1993, additional info) : Atrophy of seminiferous tubules
NOAEL < 5 mg/kg bw/d

- 2-year rat study (Kehoe, 1991a) : effect on the ovaries at the highest dose (18 mg/k bw/d)
NOAEL = 1.5 mg/kg bw/d

- Two generation reproductive toxicity in rat : NOAEL = 1.5 mg/kg bw/d (or 30 ppm)

- 13 wk rat study (Short et al, 1976 additional info) : male infertility, delayed estrous cycle

- Bird reproduction study : NOEC (Bobwhite quail, 23 wk, a.s.) = 500 mg/kg food
2/12 cases of ovary regressing at 500 and 2500 mg/kg food. Severe effects on reproduction parameters at 2500 mg/kg food

The effects on the reproduction observed in the study of Serio are in line with the effects presented in the other parts of the dossier (mammalian tox, bird reproduction). The study confirms the ADI and AOEL definitions :
ADI was based on figures of 0.84 and 1.5 mg a.s./kg/bw (lower than 6.25)
The AOEL was based on a mean NOAEL (0.84 ; 2, 3.5 mg/kgbw/d). (lower than 6.25)

The long term TER for mammals are based on the NOAEL of 1.5 mg/kg bw/d (lower than 6.25)

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

Ecological effects of Thiram 80WG in a freshwater mesocosm (Memmert, 2000)
Guidelines :
"Guidance document on testing procedures for pesticides in fresh-water mesocosms" SETAC Europe (1991)
GLP :
Material and methods :
Test substance :
THIRAM 80WG
Scenarios :
The study was performed at concentrations which would reflect spray drift scenarios of applications of thiram in orchards, grapevines or field crops.. 4 applications have been made at intervals of 7 days, the first one on 21-07-1999 and the last one on 11-08-1999.
Experimental design :
1 pond for each concentration (1, 3.2, 10, 32, 100, 320, 1000 µg a.s./l) and 3 ponds for the control
The microcosm site located in the Aachen University (Germany) consists of 10 single round tanks (diameter : 2 m; water depth : 1.6 m; sediment layer : 10 cm).

Findings :
Physico-chemical parameters in water
Oxygen content, pH and conductivity were modified at 400 and 1250 µg/l . The modification was related to algal bloom occurring after the last application.

Phytoplankton
Taxa abundance, Shannon-Weaver diversity, evenness, Steinhaus similarity and Standers similary indices were not markedly modified in the lower treatments of 1.25 to 125 µg/l and controls. The changes in the phytoplankton communities appeared to be mainly the result of indirect promoting effects due probably to the lack of grazing by zooplankton.

Macrophytes
Thiram had an inhibitory effects on Lemna at 400-1250 µg/l.

Periphyton
Effects on Periphyton algae were observed at 400-1250 µg/l

Zooplankton
Significant effects an taxa abundance were observed during the treatement period resulting in an EC20 and EC50 of 7.9 and 802 µg/l. Shannon-Weaver diversity and evenness were not affected during treatment and post-treatment period.
Pronouced effects on Steinhaus similary and Standers similarity indices were observed during the treatment and post-treatment period : EC20 of 0.2-0.3 µg/l during treatment period, EC20 of 3.8-5.9 µg/l during post-treatment period. Recovery occurs after 1-2 months for the concentrations 1.25 to 125 µg/l
Daphnia magna and *D. pulex* : EC20 during treatment period was 285 and 303 µg/l
Chydorus sphaericus, Simocephalus vetulus,: no EC calculations due to low densities, however both species were less affected than Daphnia species
Cyclopoid copepods, EC20 during treatment period was 11.1 µg/l, recovery at 1.25-400 µg/l
Calanoid copepods : no EC calculations due to low densities
Nauplii : EC20 during treatment period was 38.1 µg/l, recovery at 1.25-125 µg/l
Ostracods : not negatively affected

Rotatoria (*Brachionus , Keratella, Hexarthra*)

Keratella quadrata : EC20 during treatment period was 0.004 µg/l (biased); recovery at 1.25-12.5 µg/l
Brachionus urceolaris/variabilis : EC20 during treatment period was 10.7 µg/l, recovery at 1.25-40 µg/l.
Hexarthra mira/intermedia : EC20 during treatment period was 1.3 µg/l, recovery at 1.25-12.5g/l.

Emerging insects
No significant direct or indirect effects were observed at any test concentration.

Macro-invertebrates
- Chironomid larvae and oligochaeates were not affected by the treatment at any test concentration.
- The water snail *Gyraulus albus* was affected at the concentrations 40-1250 µg/l. EC20 and EC50 in the post treatment period were 25.1 and 25.7 µg/l. Complete recovery was not obtained during the study period.
- The leech *Helobdella stagnalis* was affected at the concentrations 125-1250 µg/l. The EC20 and EC50 in the post-treatment period were 36.4 and 40. 1 µg/l. Complete recovery was not obtained during the study period.

Conclusions :
Important effects on all taxa are observed at 125-1250 µg/l.
The author of the study reports a NOEL for the mesocosm of 1.3 µg/l and and Ecologically Acceptable Concentration of 25 µg/l or 20 µg a.s./l based on the recovery of the populations.
We consider that the effects on zooplankton were already present at concentrations < 25 µg/l (0.2-.7.9 µg/l)

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

The comparison of the author EAC (20 µg a.s./l) with the PEC showed that large buffer zones should be established in order to avoid effects on aquatic organisms. These buffer zones would not be sufficient to guarantee the absence of detrimental effects since direct toxic effects are not considered. No safety factor is considered in this evaluation.

Initial PEC in µg/l (3.2 kg a.s./ha, drift according to Ganzelmeier)

	Grapevine (early growth stage)	Grapevine (late growth stage)
3 m	52	80
5 m	17	55
7.5 m	11	27
10 m	4	18
15 m	2	9
20 m	1	4
30 m	1	2
40 m	1	-
50 m	1	-

Initial PEC in µg/l (2.4 kg a.s./ha, drift according to Ganzelmeier)

	Orchard (early growth stage)	Orchard (late growth stage)

3 m	237	124
5 m	156	80
7.5 m	113	51
10 m	85	35
15 m	50	20
20 m	34	11
30 m	16	5
40 m	3	-
50 m	2	-

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)

Assessement of the side effects of THIRAM 80 WG to honeybees, *Apis mellifera*, in the laboratory (Kling, A. ,2000)

Guidelines :
EPPO guideline No. 170 (1992)
GLP :
Yes
Material and Methods :
Test substance : THIRAM 80 WG
Test species : Honeybees (*Apis mellifera L.*); worker bees
Number of organisms : 5 groups of 10 bees
Type of test : Acute contact toxicity and acute oral toxicity test (48 hours)
Applied concentrations : 6.25, 12.50, 25, 50, 100 µg a.s. /bee (nominal) and water control
Exposure route :
- after anesthesis with CO_2 2 µl dilution of test substance solution is placed on the ventral surface of the thorax of each bee.
- A single dose of 0.25 ml to each group of 10 bees was administered
Feeding : 50 % sucrose in water ad libitum
Test conditions :
Temperature : 24-27 °C
Humidity : 50-66%
Findings :
Endpoints :
LD_{50} (Thiram 80WG, 48h) contact > 100 µg a.s. /bee (nominal)
LD_{50} (Thiram 80WG, 48h) oral > 100 µg a.s. /bee (nominal)
Conclusion :
Thiram is not toxic to bees up to 100 µg/ bee

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

Semi-field or field studies of the effects on foliage dwelling arthropods and Aphidius are required to evaluate the risk resulting from foliar spray uses .

Table B.8.5.4-1 : Summary of effects of thiram on non-target arthropods (New studies)

Species	Stage	Test Substance	Dose (kg as/ha)	Endpoint	Effect	Annex VI Trigger
Laboratory tests						
Aleochara bilineata	adults	Vitavax 200 FF	400 ml/100 kg seeds	Parasistism efficiency	21.5%	30%
Poecilus cupreus	adults	Vitavax 200 FF	400 ml/100 kg seeds	mortality	0%	30%
Aleochara bilineata	adults	Aatiram	300 g/100 kg seeds	Parasistism efficiency	28.3%	30%
Poecilus cupreus	adults	Aatiram	300 g/100 kg seeds	mortality	0%	30%
Chrysoperla carnea	larvae	Thiram 80WG	3kg/ha 6 kg/ha	Mortality mortality	87% 95%	30% 30%
Aphidius rhopalosiphi	adults	Thiram 80WG	3kg/ha 6 kg/ha 3kg/ha 6 kg/ha	Mortality Mortality Reproduction Reproduction	0% 7.69% 48% 45%	30% 30% 30% 30%

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)

Assessment of the side effects of Thiram 80WG on the activity of the soil microflora (Wachter S., 2000)
Guidelines :
BBA Guideline part VI, 1-1 (1990)
GLP :
Yes
Material and Methods :
Test Substance: THIRAM 80 WG
Soils : 3 replicates / concentration ;
1) loamy silt to loam
2) loamy to clayey sand (LUFA Speyer, 2.3)
Applied concentrations :
????? µl formulation /kg dry soil. This fortification is based on the application of 3 and 30 kg formulation/ha , an incorporation depth of 5 cm and a soil density of 1.5 g/cm
untreated control and positive control (dinoterb)

Type of test : Short-term soil respiration test (28 days)
Soil moisture : 45 and 60 % of its water holding capacity
Soil samples were incubated at 20 °C ± 2 °C in plastic bottles
Both soils were amended with glucose

Sampling scheme : 0, 14 and 28 days after treatment, aliquots were withdrawn and subjected to the respiration rate measurement
Test principle : measurement of the oxygen consumption over a period of 12 hours at different sampling intervals

Type of test : Short-term nitrogen turnover test (28 days)
Water holding capacity : 45 and 60 % of its water holding capacity
Both soils were amended with lucerne meal (0.5 % of soil dry weight)
Soil samples were incubated at 20 °C ± 2 °C, while stored in plastic bottles
Sampling scheme : 0, 14, 28, 56 and 90 days after treatment,
NH_4 - nitrogen formed from organically bound nitrogen and NO_3-nitrogen from the nitrification process were determined by using an ammonia-electrode and a nitrate-electorde, respectively

Findings :
Table 8.8.2-1: Effects on the respiration rates of 2 soils exposed to formulationTHIRAM 80 WG, over 28 days

Incubation (days)	**3 kg formulation/ha**	**30 kg formulation/ha**
	(%)	**(%)**
loamy sand, 0.5-1 %OM, pH 5.5-7.0		
	-18.6	-41.86
14	-7.89	26.32
28	-2.70	-8.11
sandy loam 1.0-3.0 %OM, pH 6.0-7.5		
	-1.26	-8.20
14	7.30	1.09
28	5.26	-4.28

Table 8.8.2-2 : Effects on the N min of 2 soils exposed to formulationTHIRAM 80 WG, over 90 days

Incubation (days)	**3 kg formulation/ha**	**30 kg formulation/ha**
	(%)	**(%)**
loamy sand, 0.5-1 %OM, pH 5.5-7.0		
	-3.32	-4.27
14	2.84	39.12
28	16.67	52.26
56	7.31	30.66
90	6.80	14.43
sandy loam 1.0-3.0 %OM, pH 6.0-7.5		
	1.08	-2.71
14	-5.49	-0.17
28	-3.32	2.22

Conclusions :
negligible effects of THIRAM 80WG on the soil respiration were observed.
negligible effects of THIRAM 80WG on the nitrogen turnover were observed (less than 15% after 90 d)

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

Activated sludge respiration inhibition test of thiram (Van der Kerken, 1999)
Guidelines :
OECD guideline N° 209
GLP :
Yes
Material and Methods :
Test Substance: thiram; purity : 99.5%
Number of replicates : 1 replicate/ concentration
Applied concentrations : 0.104, 0.329, 1.04, 3.29, 10.6, 32.8, 100.4 mg a.s./l (nominal); reference substance (3,5-dichlorophénol)
Type of test : inhibition of the respiration (3 h)
test conditions :
Incubation at 17.6-18.5°C, pH : 7.57-7.94
Findings and conclusions :
EC_{50} (3h) : 3.11 mg a.s./l (nominal)
EC_{20} (3h) : 0.59 mg a.s./l (nominal)
EC_{80} (3h) :16.2 mg a.s./l (nominal)