Sections of liver and thyroids from all animals were examined microsco-pically. At the end of the 14-week recovery period, similar necropsy, organ weights and microscopic examination were performed on all remaining rats.

13 Findings

Dosage	0, (control groups) 10, 90, 270 mg/kg/day
Clinical signs	No treatment-related clinical signs were observed
Mortality	No treatment-related deaths occurred
Body weight gain	There were no treatment-related changes in body weight gain at the dose of 10 mg/kg/day. Throughout the treatment period, moderate to marked decreases in mean body weight gain were observed in the 90 and 270 mg/kg/day groups, compared to controls.
Food consumption	There were no compound-related changes in food consumption at doses of 10 and 90 mg/kg/day and the mean compound consumption values always remained within acceptable limits in these dose groups. Throughout the treatment period, a moderate to marked increase in mean food consumption (-25% to - 48%), due to poor palatability of the medicated diets, was observed in the 270 mg/kg/day group.
T4 serum level	No treatment-related changes were observed at any dosage level
TSH and T3 serum levels	No treatment-related changes were observed at the dose of 10 mg/kg/day. Throughout the treatment period, very slight to slight decreases in mean T3 serum levels were observed at 90 mg/kg/day and 270 mg/kg/day compared to controls. These changes were associated with moderate to marked increases in mean TSH serum levels in the mid- and high-dose groups. In Recovery Week 6, most likely because of a rebound effect, serum mean TSH levels were slightly decreased at 90 and 270 mg/kg/day, compared to controls; however, these values were comparable to control values at the end of the study. In Recovery Weeks 6 and 13, a slight increase in mean T3 serum levels was observed in the 270 mg/kg/day group, compared to controls.
Liver pathology and weight	After 14 weeks of compound administration, very slight or slight increases in mean liver weight were seen in rats in the 90 and 270 mg/kg/day, respectively. Microscopically, the livers of rats in these groups had very slight or slight centrilobular hepatocellular hypertrophy.

Thyroid pathology and weight	A prominent increase in mean thyroid weights was seen in rats in the 90 and 270 mg/kg/day groups. Very slight or slight diffuse follicular cell hyperplasia was seen in most rats in these dosage groups.

Result:

A significant increase ($P < 0.05$) in thyroxine clearance was found in the 270 mg/kg/day group. In the 10 and 90 mg/kg/day groups thyroxine clearance was not significantly affected. However, in the 90 and 270 mg/kg/day groups the thyroxine volume of distribution was significantly increased ($P < 0.05$) by 22 and 64%, respectively, compared to controls. These changes in volume of distribution are probably related to the increased relative liver to body weights in these two groups. These increases in the volume of distribution without effects on clearance have the net effect of decreasing thyroxine plasma levels, resulting in compensatory increases in TSH serum levels. TSH has been shown in a variety of models to act as a growth stimulator and tumor promotor for the rat thyroid gland, but not for most other species, including humans.

Therefore, these data support the conclusion that thiabendazole affects the thyroid of rats indirectly by altered thyroxine clearance via increased hepatic metabolism. This mechannism is specific to rats and does not result in an increased risk to humans since alterations in thyroid homeostasis do not produce increases in thyroid tumors in humans (Hill et al., *Fund. and Appl. Toxicol.*, 12 (1989), 629-697 [included with the report in the K documents]).

Conclusion:

Administration of thiabendazole induced very slight and slight decreases in T3 serum levels associated with moderate to marked increases in TSH serum levels at 90 and 270 mg/kg/day, respectively. TSH serum levels returned to control values at the end of the study after a transient decrease in Recovery Week 6 observed in both groups. In Recovery Weeks 6 and 13, T3 serum levels were slightly increased at 270 mg/kg/day. These changes were associated with marked increases in liver and thyroid weight and thyroid follicular cell hyperplasia after 14 weeks of thiabendazole treatment. All of these changes were reversible after 14 weeks of recovery.

Based on the treatmennt-related alterations in thyroxine clearance in the 90 and 270 mg/kg/day groups, these thyroid changes are considered indirect effects of thiabendazole treament related to a hypothyroid condition. Such changes are specific to rodents and are not relevant to human for risk assessment. In addition, these results are consistent with the previously conducted thiabendazole rat carcinogenicity study (see 5.5/02) where the NOEL for thyroid adenomas was 10 mg/kg/day i.e. the NOEL for alterations in thyroid homeostatis was 10 mg/kg/day.

14 Statistics

Snedecor G.W. and Cochran W.G. (1989), "Statistical Methods", 8th Edition, Iowa State University Press; pp 290-291.

Mood, A., Graybill F. and Boes D. (1974), "Introduction to the Theory of Statistics", 3rd Edition, McGraw-Hill, New York; p 181.

Snedecor G.W. and Cochran W.G. (1989), "Statistical Methods", 8th Edition, Iowa State University Press; pp 217-236.

Tukey, J.W., Ciminera, J.L. and Heyse, J.F. (1985), "Testing the Statistical Certainty of a Response to Increasing Doses of a Drug", Biometrics, vol. 41, pp. 295-301.

15 References to

	publications	R.N. Hill et al., Fundamental and Applied Toxicology, 12, 629-697, 1989. Oppenheimer, J.H., Bernstein, G., Surks, M.I. Increased Thyroxine Turnover and Thyroidal Function after Stimulation of Hepatocellular Binding of Thyroxine by Phenobarbital. J. Clinical Investigation, 1968; 47: 1399-1406.
16	**Unpublished data**	not applicable

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2005
Materials and Methods	The study complied with GLP and the laboratory is subject to US EPA inspection
	[redacted]
Results and discussion	[redacted]

[redacted]

Conclusion [redacted]

Reliability [redacted]

Acceptability [redacted]

Remarks

98/8 Doc IIIA section No.	6.11	Studies on other routes of administration (parenteral routes)

Not applicable

98/8 Doc IIIA section No.	6.12	Medical data

Data is available from years of production; use as a pharmaceutical compound, but considered not applicable here

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2005
Materials and Methods	
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	6.13	Toxic effects on livestock and pets

[redacted]

98/8 Doc IIIA section No.	6.14	Other test(s) related to the exposure of humans

[redacted]

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	6.15	Food and feedingstuffs

[redacted]

[redacted]

98/8 Doc IIIA section No.	**6.16**	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**

[redacted]

RMS: [redacted]

98/8 Doc IIIA section No.	**6.17**	**If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required**

[redacted]

RMS: [redacted]

98/8 Doc IIIA section No.	6.18	Summary

A summary of the Mammalian Toxicology and Conclusions for the active substance thiabendazole is provided in Document IIA

COMPETENT AUTHORITY REPORT

THIABENDAZOLE (PT8)

DOCUMENT III-A
Active Substance

Section 7: Ecotoxicological Profile Including Environmental Fate and Behaviour

Rapporteur Member State: Spain

April 2006

Fate and behaviour in water

98/8 Doc IIIA section No.	**7.1.1.1.1 / 01**	**Hydrolysis as a function of pH and identification of breakdown products**
91/414 Annex Point addressed	**II 7.2.1.1 / 01**	**Rate of hydrolysis**

1.2	**Title**	Hydrolysis as a Function of pH at 25°C of ^{14}C-Thiabendazole
1.3	**Report No.**	37636
1.4	**Lab. report No.**	not applicable
1.5	**Cross reference**	7.2.1.1/01
1.6	**Authors**	Kent Kabler, Chemist I and John Dykes, Chemist III
1.7	**Date of report**	26 July 1989
1.8	**Published**	no
2.1	**Testing facility**	Analytical Bio-Chemistry Laboratories, Inc., P.O. Box 1097, Columbia, MO 65205, USA
2.2	**Dates of experimental work**	20 January 1989 to 10 March 1989
3	**Objective**	to determine the hydrolysis rate constants, half-lives for degradation and characterize the major hydrolysis products of Thiabendazole as a function of pH
4.1	**Test substance**	Thiabendazole [2-(4-thiazolyl)-1H-benzimidazole] Composition: uniformly labelled with ^{14}C in the phenyl ring with a specific activity of 24.77 microcuries/mg and radiochemical purity of [redacted] [^{14}C]thiabendazole
4.2	**Specification**	[redacted]
4.4	**Stability in vehicle**	not applicable
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle/solvent**	buffered water solutions
6	**Physical form**	powder
7.1	**Test method**	Study design follows EPA/FIFRA subdivision N guidelines 161-1, 1982
7.2	**Justification**	internationally accepted method
7.3	**Copy of method**	description of methods included in report
8	**Choice of method**	not applicable
9	**Deviations**	the test protocol deviated from the OECD guideline #111 in terms of pH levels (3 instead of 4 different levels) and temperature (1 instead of 2). These differences did not affect the validity of the test conclusions, as the material was found to be stable under all conditions.
10.1	**Certified laboratory**	not applicable
10.2	**Certifying authority**	not applicable
10.3	**GLP**	yes
10.4	**Justification**	not applicable

11.1 GEP not applicable

11.2 Type of facility (official or officially recognized) not applicable

11.3 Justification not applicable

12 Test system

Test containers: Pyrex culture tubes (15 ml) with teflon-lined screw caps were used as sample containers

Test concentrations: the hydrolysis study was conducted at a nominal test concentration of 10.0 mg/l in 4 aqueous buffer solutions [pH 5, 0.1 M acetate, pH 7, 0.1 M TRIS (hydroxymethyl) aminomethane {TRIS}, pH 7, 0.01 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid {HEPES*}, and pH 9, 0.1 M borate buffer].

Study conditions: the study was conducted in the dark

Sampling: samples were taken 0, 1, 3, 7, 14, 21 and 30 days after initiation.

Radioanalysis: confirmation of percent [^{14}C]Thiabendazole in each test sample was achieved using high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Liquid scintillation counting was used to determine the total activity of test compound at each sample point.

13 Findings

Rate constant and half-lives determined for each test system		
System	Rate constant (k)	Half-life
pH 5	0.0019 days^{-1}	357.1 days
pH 7 (HEPES)	0.0034 days^{-1}	203.0 days
pH 7 (TRIS)	***	***
pH 9	0.0026 days^{-1}	270.8 days

*** positive slope - no degradation

Conclusion: Based on the data generated in this hydrolysis study (pH 5-9; 25^{o}C; 10 micrograms/ml), [^{14}C]thiabendazole is hydrolytically stable (half-lives of 357, 203 and 271 days at pH values of 5, 7 [HEPES] and 9, respectively). After 30 days the rate of degradation was not statistically different than zero at pH 5, and 91 and 87.8% of the parent compound remained at pH values of 7 [HEPES] and 9, respectively.

14 Statistics results of the linear regression analysis were checked for significance by use of the standard t test.

15 References to publications

H. Suffet, C. W. Carter and G. T.Coyle, "Hydrolysis Protocols. Effects of Water on the Environmental Fate of Chemicals," in "Test Protocols for Environmental Fate and Movements of Toxicants", A.O.A.C. Symposium Proceedings, 94th Annual Meeting, October 21-22, 1980, Washington, DC.

* also referred to as 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid

16 Unpublished data not applicable

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9-3-2004
Reference	Kabler, K. and Dykes, J. (1989) Stability in Water: Hydrolysis as a Function of pH at 25 °C of [^{14}C]-Thiabendazole. Document IIIA_sec 7b submitted by applicant: 7.1.1.1.1/01 Document IVA_sec submitted by applicant: 7.1.1.1.1.
Materials and Methods	[redacted]
Results and discussion	[redacted]

Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.1.1.1.1 / 02**	**Hydrolysis as a function of pH and identification of breakdown products**
91/414 Annex Point addressed	**II 7.2.1.1 / 02**	**Rate of hydrolysis of relevant metabolites**

not applicable due to stability of parent

98/8 Doc IIIA section No.	**7.1.1.1. 2 / 01**	**Phototransformation in water including identitiy of products of transformation**
91/414 Annex Point addressed	**II 7.2.1.2 / 02**	**Direct phototransformation**

1.2	**Title**	Determination of the Aqueous Photolysis Rate of [^{14}C]Thiabendazole
1.3	**Report No.**	41285
1.4	**Lab. report No.**	not applicable
1.5	**Cross reference**	7.2.1.2/01
1.6	**Authors**	Joe Flynn, Chemist II/Study Director
1.7	**Date of report**	20 July 1994
1.8	**Published**	no
2.1	**Testing facility**	Analytical Bio-Chemistry Laboratories, Inc., Environmental Fate and Assessment, 7200 E. ABC Lane, Columbia, MO 65202-8015, USA
2.2	**Dates of experimental work**	15 November 1993 to 5 April 1994
3	**Objective**	to determine the photolytic half-life for the test substance, [^{14}C]Thiabendazole, in pH 5 buffer and to identify photodegradates present at a concentration greater than 10% of initial measured dose (IMD)
4.1	**Test substance**	Thiabendazole [2-(4-thiazolyl)-1H-benzimidazole] Composition: uniformly labelled with ^{14}C in the phenyl ring with a specific activity of 57.40 microcuries/mg and radiochemical purity of [redacted] [^{14}C]Thiabendazole
4.2	**Specification**	[redacted]
4.4	**Stability in vehicle**	not applicable
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle/solvent**	methanol and 0.1 M pH 5 acetate buffer
6	**Physical form**	powder
7.1	**Test method**	Study design follows EPA/FIFRA subdivision N guidelines 161-1, 1982
7.2	**Justification**	internationally accepted method
7.3	**Copy of method**	description of methods included in report (check)
8	**Choice of method**	not applicable
9	**Deviations**	not applicable
10.1	**Certified laboratory**	not applicable
10.2	**Certifying authority**	not applicable
10.3	**GLP**	yes
10.4	**Justification**	not applicable
11.1	**GEP**	not applicable
11.2	**Type of facility (official or officially recognized)**	not applicable
11.3	**Justification**	not applicable
12	**Test system**	

Duration of test: 96 hours

Test concentration: the study was conducted at a nominal test concentration of 10 micrograms/ml (10 ppm) in 0.1 M pH 5 acetate buffer at 25°C

IMD: 9.36 µ/ml (9.36 ppm)

Lighting: test samples were placed in quartz glass tubes and exposed to light from a xenon arc lamp for 96 hours

Sampling: performed at 0, 6, 12, 18, 24, 36, 48, 72 and 96 hours of exposure

Radioanalysis: the % [^{14}C]-Thiabendazole in each test sample was determined using high pressure liquid chromatography (HPLC). Liquid scintillation counting was used to determine the total ^{14}C-activity at each sample point and to quantitate radioactivity in HPLC fractions.

13 Findings

Half-life estimation - exposed system			
Sample interval (Hours)	% Thiabendazole (of Time 0)	LN of percent TBZ (of Time 0)	Estimate of y for regressed line
0	100.00	4.61	4.61
6	90.93	4.51	4.74
12	78.47	4.36	4.33
18	68.14	4.22	4.18
24	57.37	4.05	4.04
36	42.94	3.76	3.75
48	26.86	3.29	3.47
72	16.77	2.82	2.89
96	11.46	2.44	2.32

Regression data: (see report)

X coefficient (m): ..... -0.0239
Constant (b): 4.6132
Standard error of Y estimate: 0.0888
Standard error of X coefficient estimate: 0.0010
Number of observations: 9
Degrees of freedom: 7
Correlation coefficient (r): 0.994
Photolysis rate: ..... 0.0239 hour^{-1}
Half-life: ..... 29.0 hours

Note: values were not rounded before final half-life calculation

Results:

The test chemical, [^{14}C]thiabendazole, degraded under the test conditions. The calculated half-life of [^{14}C]thiabendazole in pH 5 buffer was 29.0 hours with a photolysis rate constant of 0.0239 hour^{-1}. The correlation coefficient for the half-life regression analysis (r) was 0.994. Mean mass accountability for the exposed study samples was 102 $\pm$ 4.14%. Mean mass accountability for the nonexposed study samples was 102 $\pm$ 1.28%. Sterility was maintained in both exposed and nonexposed samples during the course of the study as indicated by the absence of microbes on agar plates. There was no degradation apparent in the nonexposed control samples.

At least 7 distinct radioactive (hence TBZ related) chromatographic regions, in addition to parent, were observed in degraded samples. Only one of the degradate regions contained more than 10% of IMD, and it was identified by HPLC as [^{14}C]benzimidazole-2-carboxamide (10.22% of IMD). A second degradate, [^{14}C]benzimidazole, which was 6.49% of IMD was also identified by HPLC. The identity of these two degradates and [^{14}C]thiabendazole was confirmed by mass spectrometry.

Conclusion:

Based on the results of this study, it can be concluded that Thiabendazole in aqueous solution will undergo photolytic degradation, following first-order kinetics, in the presence of sunlight with a half-life of approximately 29 hours. Photodegradation will primarily involve structural alteration of the thiazole ring. Formation of at least seven photodegradates can be expected, with benzimidazole-2-carboxamide as the major product. Lesser amounts of benzimidazole (and possibly benzimidazole-2-carboxylic acid, based only on HPLC data) can be expected to be produced.

14	**Statistics**	no statistical analysis was carried out, as it was considered unnecessary for interpretation of the results and therefore not required.
15	**References to publications**	Lloyd A. Curie, 1968, "Limits for Qualitative Detection and Quantitative Determination; Application to Radiochemistry," Analytical Chemistry, 40, 586-593.
16	**Unpublished data**	not applicable

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9-7-2004
Reference	Flynn, J. (1994) Determination of the Aqueous Photolysis Rate [^{14}C]-Thiabendazole. Document IIIA_sec 7b submitted by applicant: 7.1.1.1.2/01 Document IVA_sec submitted by applicant: 7.1.1.1.2.2.
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.1.1.1.2 / 02**	**Phototransformation in water including identitiy of products of transformation**
91/414 Annex Point addressed	**II 7.2.1.2 / 02**	**Direct phototransformation**

Quantum Yield of Direct Phototransformation of Thiabendazole

Report: Schmidt, E. (2002): Quantum Yield of the Direct Photochemical Degradation of Thiabendazole in Aqueous Solution. Solvias AG, Physical Chemistry, CH – 4002 Basel, Switzerland. Unpublished report, Proj.No L01-008389, 17 April, 2002.

Guideline: OECD Environmental Health and Safety Publications, Series on Testing and Assessment, No. 7, "Guidance Document on Direct Phototransformation of Chemicals in Water", Paris 1997.

OPPTS 835.2210, "Direct Photolysis Rate in Water by Sunlight", Fate, Transport and Transformation Test Guidelines, EPA, January 1998.

OECD Guideline for Testing of Chemicals; Proposal for a New Guideline "Phototransformation of Chemicals in Water - Direct and Indirect Photolysis", Draft Document, August 2000.

Zepp, R.G., Cline D.M., "Rates of Direct Photolysis in Aquatic Environment", Environmental Science and Technology, 11, 359 (1977).

Council Directive 94/37/EEC, Annex I, 22 July 1994, § 2.9.2, amending Council Directive 91/414/EEC, 15 July 1991.

OECD Guideline for testing of Chemicals, 101; "UV-Visible Absorption Spectra"; Paris, France; OPPTS 830.7050; EPA, August 1996

GLP: yes (A.Sutter, Quality Assurance Consulting AG, CH – 4431 Bennwil, Switzerland)

Test system: The spectra and the direct photolysis quantum yields of thiabendazole [redacted] (pK_{a1} = 4.73, pK_{a2} = 12.00) were determined in dilute neutral and acidic aqueous solutions at pH-values of ca 7.35 and 2.75 for the predominantly neutral and the protonated form, respectively, with 10% acetonitrile added as co-solvent. Test concentrations were ca 7.7 x 10^{-7} mol/l (ca 0.155 mg/l) and ca 8.08 x 10^{-7} mol/l (ca 0.163 mg/l) in the neutral and the acidic test solutions, respectively. Irradiation occurred in two independent test series with light of 300 nm in rectangular cuvettes of Suprasil quartz glass with optical pathlength of 1.0 cm (test conditions are given in Table 9.2 – 1). The irradiated volume was 2.0 ml. The cuvettes were equipped with magnetic stirrers and were tightly stoppered. The dark controls were identically prepared. Temperature was kept at 21 ± 0.5°C by a water cooled thermostat. For irradiation a spectrofluorimeter "SPEX Fluorolog" was used. It was equipped with a 450 W high pressure Xenon-lamp and a double monochromator with a dispersion of 1.8 nm/mm. The incident light flux was determined radiometrically. Quantification of the phototransformation process of thiabendazole was performed by HPLC analysis. Samples were stored in a refrigerator until HPLC analysis for identification and quantification of thiabendazole was performed. Company code No. CGA 28020 mentioned in some tables of the report is identical with the code MK 360 = thiabendazole.

Irradiation Conditions for Test Solutions of Thiabendazole (Schmidt 2002)

	predominantly neutral form	**predominantly protonated form**
Solvent	phosphate buffer 0.01 M, pH = 7.35	'Veibel' buffer diluted to pH = 2.75
Measured thiabendazole concentration [mg/l]	ca 0.155	ca 0.163
Irradiation wavelength [nm]	300	300
Slit width [mm]	5	5
Irradiation half-width [nm]	9	9
Si-diode reading [µA]	50.35± 0.03	49.19 ± 0.37
Diode-Sensitivity [A/W]	0.040275	0.040275
Irradiation times [min] for series 1 and 2	0, 30, 60, 120, 180	0, 30, 60, 120, 180

The quantum yields (as the ratio of the number or moles of molecules undergoing photolysis to the number or moles of photons absorbed) were calculated by using the phototransformation rate constants (obtained from the ratio of thiabendazole concentrations in irradiated solutions and dark controls for the different irradiation times based on the two experimental runs using first order decay kinetics; the mean half-lives being ca 1.93 and 2.1 hours for the neutral and the protonated form, respectively), the incident and absorbed light flux of the xenon light source (obtained by radiometric evaluation), the irradiated volume of the test substance and the molar absorption coefficients at the irradiation wavelength (obtained by UV-spectroscopy). The half-lives for direct photolysis in shallow surface waters were calculated for spring and summer season with the program GCSOLAR for several geographical latitudes in the pH range 5 to 9, hence the program considers spectra and quantum yields of direct photolysis and calculates half-life values in representative aquatic systems.

Findings: The adsorption spectrum of the neutral form of thiabendazole in aqueous solution had a long wavelength offset at 350 nm and a maximum at 298 nm (molar absorption coefficient ε = 22470 L/mol/cm). In acidic solution the offset was 390 nm with the maximum found at 302 nm (ε = 25550 L/mol/cm). The experimental incident photon fluxes on the cuvettes as determined by radiometry amounted to approximately 3×10^{-9} mol/s at 300 nm for both the neutral and the acidic test solution.

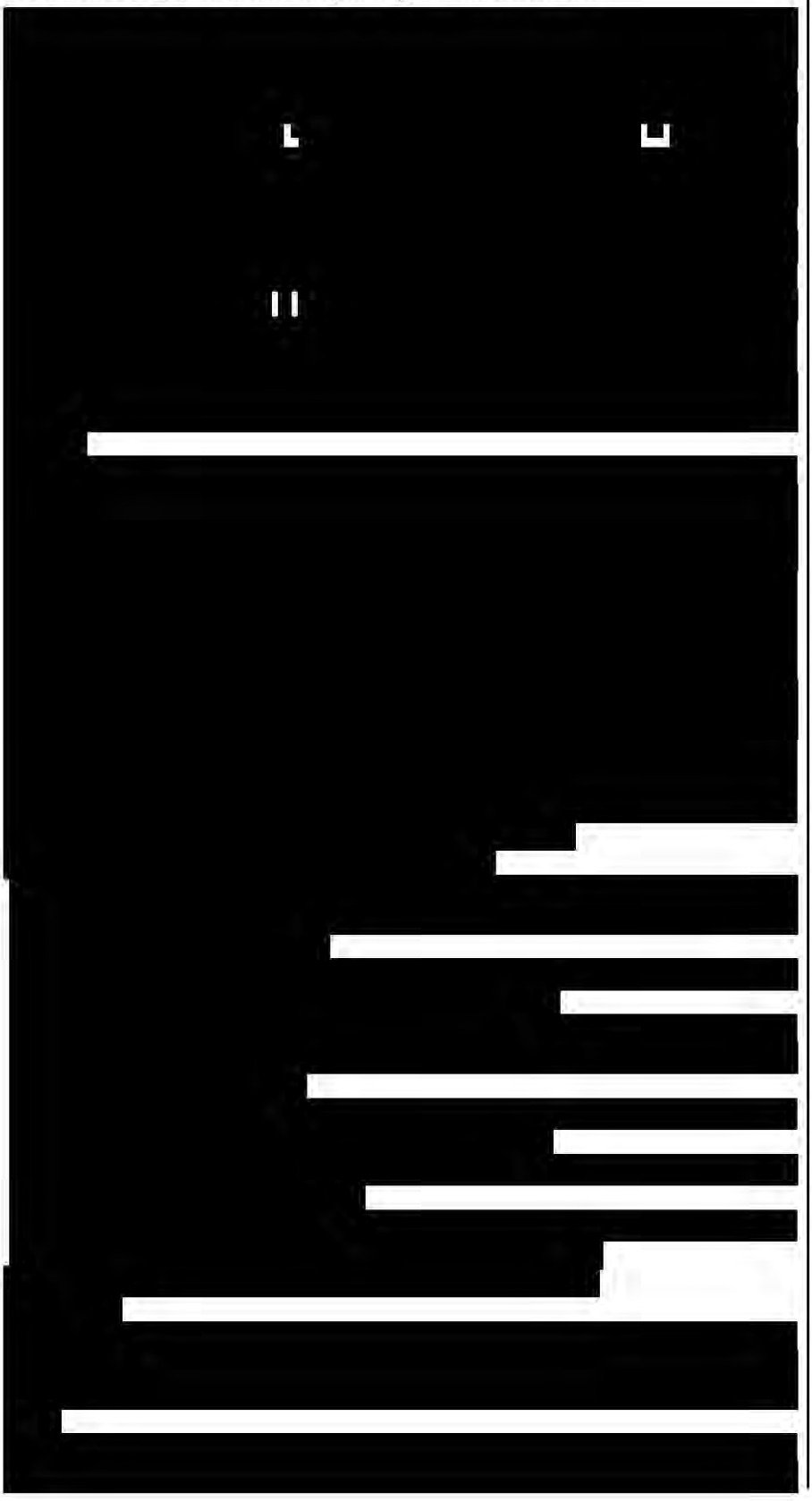
The kinetic parameters of the laboratory phototransformation are summarised in Table 9.2 – 2. The test substance concentration in dark controls was practically constant, i.e. no dark reaction occurred. Average quantum yields of $\Phi = 0.00136 \pm 0.00002$ and $\Phi = 0.00110 \pm 0.00002$ were determined in neutral and acidic solutions, respectively. The photolytic half-lives of thiabendazole in pure shallow waters over a full day of 24 hours were calculated for spring and summer at geographical latitudes of 30° N , 40° N and 50° N corresponding to locations such as Kairo or New Orleans, Madrid or Denver and Winnipeg or Frankfurt, respectively. Half-lives between 0.68 days (30° N, summer) and 1.52 days (50° N, spring) were estimated for the neutral molecule and half-lives between 0.44 and 0.87 days for the protonated form, respectively.

Kinetics of Phototransformation and Estimations of Environmental Photolytic Half-lives of Thiabendazole (Schmidt 2002)

		predominantly neutral form	**predominantly protonated form**
Mean rate constant k_p [s^{-1}]:		9.87×10^{-5}	9.18×10^{-5}
Mean half-life DT_{50} [h]:		1.93	2.1
Quantum yield		0.00136	0.00110
Environmental half-lives* [days]:			
Latitude 30°N	Spring	0.83	0.52
	Summer	0.68	0.44
Latitude 40°N	Spring	1.07	0.65
	Summer	0.76	0.48
Latitude 50°N	Spring	1.52	0.87
	Summer	0.89	0.55

*Calculated with the program GCSOLAR

From the results it can be concluded that thiabendazole is rapidly degraded by direct sunlight in natural waterbodies with half-lives ranging from ca 0.5 to 1.5 days at latitudes 30° N, 40° N or 50° N. Photolysis under acidic conditions proceeds at a higher rate than in neutral solutions; apparently, the increased absorption of sunlight overcompensates the lower quantum yield as compared to the neutral molecule. Overall, direct phototransformation in aqueous systems is considered to be a relevant process for the lifetime of thiabendazole when released into an aqueous environment. The results are in quite a good agreement with the findings of the aqueous photolysis study with thiabendazole (*Flynn 1994*), where a photolytic half-life of 29 hours (= 1.2 days) was reported (see Annex IIA, section 5).

	Evaluation by Competent Authorities
	EVALUATION BY THE RAPPORTEUR MEMBER STATE
Date	5-5-2005
Reference	Schmidt, E. (2002) Quantum Yield of the Direct Photochemical Degradation of Thiabendazole in Aqueous Solution. Document IIIA_sec 7b submitted by the applicant: 7.1.1.1.2/02. Document IVA_sec 7 submitted by the applicant: 7.1.1.1.2.3.
Materials and Methods	
Results and discussion	

absorption coefficient ε = 22470 L/mol/cm). In acidic solution the offset was 390

Conclusion

Reliability

Acceptability

Remarks

98/8 Doc IIIA section No.	**7.1.1.2.1 / 01**	**Ready biodegradability**
91/414 Annex Point addressed	**II 7.2.1.3.1 / 01**	**Ready biodegradability**

98/8 Doc IIIA section No.	**7.1.1.2.1 / 02**	**Ready biodegradability**
91/414 Annex Point addressed	**II 7.2.1.3.1 / 01**	**Ready biodegradability**

Ready Biodegradability

Report: Van der Kolk, J. (1998a): MK 360 (Thiabendazole) ready biodegradability CO_2 evolution test (modified Sturm test). Springborn Labs., CH - 9326 Horn, Switzerland. Unpublished report, Proj.No 98-229-1047/1047.025.775, 24 September 1998.

Guideline: OECD guideline 301B (1992b).

GLP: yes (Springborn Labs. Europe, Quality Assurance, CH – 9326 Horn)

Test system: The test was performed with thiabendazole technical grade of [redacted] purity (carbon content 59.6% based on the empirical formula $C_{10}H_7N_3S$, molecular weight 201.3) in a mineral medium inoculated with activated sludge collected from a sewage treatment plant. The test system is described in Table 9.2 – 3. During incubation the evolved carbon dioxide was measured at 0, 1, 2, 3, 6, 14, 20, 24 and 30 days. Several test flasks were set up: two flasks containing thiabendazole plus sludge inoculum, two flasks as inoculum blank without thiabendazole, one flask for the reference compound sodium benzoate plus inoculum without thiabendazole, one flask for abiotic sterile control and one flask for toxicity control containing thiabendazole, sodium benzoate plus inoculum. The test vessels were aerated with carbon dioxide free air.

Degradation was determined from the amount of CO_2 produced measured via $Ba(OH)_2$ precipitation in the absorber flasks at the respective sampling intervals. The theoretical CO_2 amount ($ThCO_2$), if all total organic carbon (TOC) would be respired, was calculated based on the TOC at test initiation. The conversion factor between $ThCO_2$ and the TOC per test flask was taken as 3.67 (= MW ratio carbon doixide/carbon = 44/12 = 3.67), hence

$ThCO_2 = 3.67 \times TOC$ [mg].

The biodegradability of the compound was then expressed as the percentage of the actual amount of CO_2 produced in each flask (under consideration of the carbon dioxide production in the blank test vessels which had to be subtracted first) divided by the $ThCO_2$.

Test system for carbon dioxide evolution study (Van der Kolk1998a)

pH of test solutions	ca 7.4
Test volume per flask [L]	3
Source	Sewage treatment plant, Romanshorn, Thurgau, Switzerland
Test concentration [mg/L]	ca 16.9, corresponding to 10.1 mg TOC/L*
Total $ThCO_2$* in test flasks	- ca 111 mg in flasks from thiabendazole, where present - ca 219.5 mg in flasks from sodium benzoate, where present
Duration	30 days
Temperature	22 ± 2°C, with some short-time deviations
Reference substance	Sodium benzoate, ca 50% carbon (MW = 144.1 g/mole)

*TOC = Total Organic Carbon; $ThCO_2$ = Theoretical amount of carbon dioxide

Findings: The biodegradation of thiabendazole accounted for 6.5% of the theoretical value within 30 days in one flask while it was below the values of the blank controls in the other flask containing thiabendazole (ca 20% less at the end of incubation). The reference substance was degraded to 79% in the same period. According to the 7th Amendment to Directive 67/548/EEC, i.e. Directive 92/32/EEC, thiabendazole is therefore classified as not readily biodegradable although the amount of carbon dioxide produced in one flask illustrates at least the potential for

a slow mineralisation of the test substance. The sterile flask results demonstrated further that no abiotic degradation (hydrolysis) took place under the selected test conditions. The toxicity control flask results gave clear evidence that thiabendazole did not adversely affect the degradation of the reference substance sodium benzoate, since the CO_2 evolution was comparable to that one in the pure reference substance flask without the addition of thiabendazole

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09-15-2004 Van der Kolk, J. (1998a)
Reference	MK 360 (Thiabendazole) ready biodegradability CO_2 evolution test (modified Sturm test). Document IIIA_sec 7b submitted by the applicant: 7.1.1.1.2/02. Document IVA_sec submitted by the applicant: 7.1.1.2.1.
Materials and Methods	[redacted]
Results and discussion	[redacted]

[redacted]

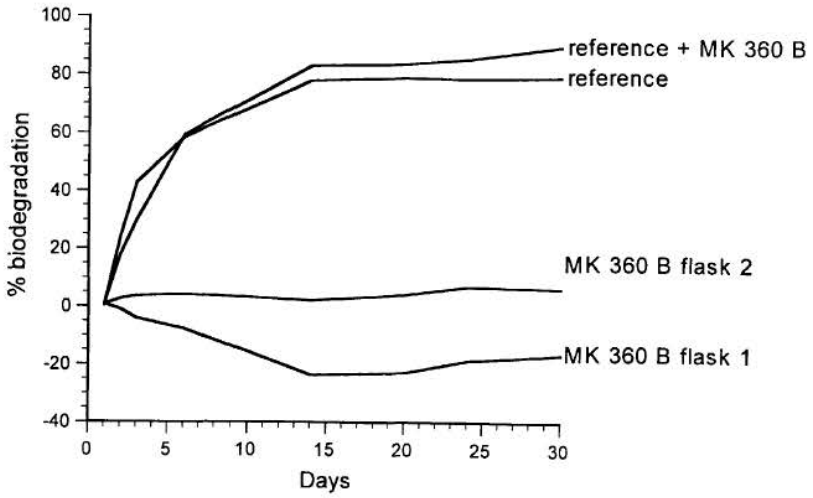
Conclusion
Reliability

Acceptability
Remarks

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Figure 1. **Percent biodegration of MK 360 B and sodium benzoate, based on the theoretical amount of CO_2 added at test initiation and the amount of CO_2 evolved.**



98/8 Doc IIIA section No.	**7.1.2 / 02**	**Rate and route of degradation in aquatic systems including identification of metabolites and degradation prodcuts**
91/414 Annex Point addressed	**II 7.2.1.3.2 / 01**	**Water / sediment study**

Water/Sediment Studies with Thiabendazole

1.2	**Title**	Biodegradation of Thiabendazole in an Aerobic Water/Sediment System
1.3	**Report No.**	R88/166
1.4	**Lab. report No.**	MTB-238
1.5	**Cross reference**	7.2.1.3/01
1.6	**Authors**	J.W. Vonk, Study Director
1.7	**Date of report**	5 September 1988
1.8	**Published**	no
2.1	**Testing facility**	TNO Division of Technology for Society, P.O. Box 217, 2600 AE Delft, The Netherlands
2.2	**Dates of experimental work**	19 January 1988 to 12 April 1988
3	**Objective**	as title
4.1	**Test substance**	Thiabendazole [2-(4-thiazolyl)-1H-benzimidazole] active substance unlabeled as manufactured and active substance labeled in the laboratory
4.2	**Specification**	Production lot No. [redacted], a.i. specification [redacted], unlabeled technical material Lot # [redacted], a.i. specification approximately [redacted] uniformly labeled with the ^{14}C in the phenyl ring with a specific activity of 26.4 microcuries/mg
4.3	**Storage stability**	stability of labeled product before and after test period was determined by manufacturer and found to be the same
4.4	**Stability in vehicle**	material was dissolved in small amounts of DMSO prior to dilution in water and the concentration monitored throughout test period. Stable. The amount of dissolved material was consistent with the measured concentrations throughout the test period.
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle/solvent**	DMSO/water
6	**Physical form**	powder
7.1	**Test method**	Study design follows Dutch guidelines G.2.1
7.2	**Justification**	not applicable
7.3	**Copy of method**	description of methods included in report and described in section 12
8	**Choice of method**	not applicable
9	**Deviations**	not applicable

10.1 Certified laboratory not applicable

10.2 Certifying authority not applicable

10.3 GLP no

10.4 Justification GLP was not in effect in the Netherlands at the time the study was conducted

11.1 GEP not applicable

11.2 Type of facility (official or officially recognized) internationally recognized test laboratory

11.3 Justification not applicable

12 Test system

Soil/water source: TNO-Zuidpolder ditch water in the Delft area, with a sediment sample from the 5 cm top layer of the same ditch. Second sediment sample taken from the river "Kromme ijn" near Odijk, Netherlands. The content of organic matter of the TNO sediment is higher than that of the "Kromme Rijn".

Analysis: sediment samples were analyzed for their pH and their content of organic matter, calcium carbonate, silt, clay and sand. The sediments were allowed to settle and were then sieved to remove coarse particles before being added to the test flasks

Dry weights: determined by drying subsamples at 105°C for about 17 hours

Test conditions: test carried out in cylindrical biometer flasks, closed with screw caps from which CO_2 traps (scintillation vials) were suspended. 100 ml of ditch water + a sufficient amount of wet sediment to give a 1% content of dry solids was placed in each flask.

Duration of test: 12 weeks

TBZ concentrations: 1.0 (test run in duplicate) and 0.3 mg (test run single) of labeled and unlabeled TBZ/l.

pH: of ditch water was 8.2

Oxygen level: refreshed every 2 weeks

CO_2 traps: collected after 1, 2, 4, 6, 8, 10 and 12 weeks and radioactivity determined (scintillation)

Analytical procedures: biodegradation was determined by monitoring the evolution of ^{14}C-CO_2 as a measure of ultimate biodegradation, by determining the radioactivity present in the aqueous phase and in the extracts of the sedi-ments and by characterization of the radioactive compounds present.

TBZ and its potential metabolites were determined in the aqueous phases and the organic extracts of the solids by TLC, if radioactivity exceeded 5% of the initial activity.

13 Findings

Biodegradability of 1.0 mg/l Thiabendazole.
The figures represent the % recoveries of the initial amounts of radioactivity added

Time	replicate 1					replicate 2				
weeks	CO_2	H_2O	Sol.	Res.	Rec.	CO_2	H_2O	Sol.	Res.	Rec.
"Kromme Rijn" water/sediment system										
0	-	80	13	2	95	-	84	13	3	100
1	0	71	16	6	93	0	78	18	6	102

2	0	73	20	8	101	0	74	19	8	101
4	0	42	26	10	78	0	44	23	8	75
6	0	64	21	15	100	0	63	24	14	101
8	0	60	21	18	99	0	61	22	15	98
10	1	64	19	17	101	1	62	21	15	99
12	1	61	21	20	103	67	2	2	23	94

Biodegradability of 1.0 mg/l Thiabendazole. The figures represent the % recoveries of the initial amounts of radioactivity added										
Time	replicate 1					replicate 2				
weeks	CO_2	H_2O	Sol.	Res.	Rec.	CO_2	H_2O	Sol.	Res.	Rec.
TNO water/sediment system										
0	-	41	38	16	95	-	42	41	17	100
1	0	24	50	27	101	0	23	50	32	105
2	0	20	50	32	102	0	23	49	32	104
4	0	33	51	28	112	0	33	47	34	114
6	1	16	46	30	93	1	16	46	33	96
8	1	13	40	43	97	1	13	42	57	113
10	1	14	38	42	95	1	15	43	43	102
12	1	15	42	55	113	1	15	41	48	105

Legend:
CO_2 : radioactivity collected in the carbon dioxide trap
H_2O : radioactivity in the aqueous phase
Sol. : extractable radioactivity in the solids
Res. : residual radioactivity in the solids (bound residue)
Rec. : recovery

Results: the amount of Thiabendazole decreased from 90% to 80% in the organic and aqueous extracts of the solids for the "Kromme Rijn" soil and from 77 to 55% for the "TNO-water/sediment" system. The same 3 unidentified radioactive spots of low concentration (Rf's equal to 0.8, 0.3 and 0.0) were defined in both systems, none of which corresponded to the 5-OH-Thiabendazole standard with a Rf of 0.5.

Amounts of Thiabendazole and other radioactive substances as percentages of the initial radioactivity in both water/sediment systems								
Time	'Kromme Rijn' water/sediment system				TNO water/sediment system			
(weeks)	MA	0.8	0.3	0.0	MA	0.8	0.3	0.0
0	90	1	1	2	77	<1	<1	1
1	83	1	1	1	73	1	<1	<1
2	89	1	1	2	69	<1	<1	<1
4	66	<1	<1	1	82	1	1	1
6	82	1	1	2	60	<1	<1	1
8	78	1	1	2	51	<1	<1	1
10	80	1	<1	2	50	<1	<1	1
12	79*	<1	<1	2	55	<1	<1	1

MA: parent compound (Thiabendazole)
0.8, 0.3, 0.0: Rf-values of the radioactive substances
* In replicate 2 with increased mineralization Thiabendazole was probably <4%

	Conclusion:	Thiabendazole appeared to be metabolized in both the high and low organic content water/sediment systems tested in the Netherlands. However, the identity of the radioactive spots detected by TLC did not correspond to the 5-OH-Thiabendazole metabolite found in animal metabolism studies. Due to the very low concentration of these materials and the fact that Thiabendazole tended to tail in the solvent system used, the possibility exists that these materials are artifacts.
14	**Statistics**	no statistical analysis was carried out, as it was considered unnecessary for interpretation of the results and therefore not required.
15	**References to publications**	none

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09-21-2004
Reference	J.W. Vonk. (1988) Biodegradation of Thiabendazole in an Aerobic Water/Sediment System. Document IIIA_sec 7b submitted by applicant: 7.1.1.2.1 / 01 Document IVA_sec submitted by applicant: -

Materials and Methods

[redacted]

Results and discussion

[redacted]

Conclusion

Reliability

Acceptability

Remarks

Report: Ulbrich, R. (1999): MK 360 (CGA 28020): Degradation and Metabolism of ^{14}C-Phenyl-Labelled MK 360 in Two Aerobic Aquatic Systems under Laboratory Conditions. Novartis Crop Protection AG, Environmental Safety, Ecochemistry, CH – 4002 Basel, Switzerland. Unpublished report, Proj.No 98UL06, 13 December 1999.

Guideline: Commission Directive 95/36/EC of 14 July 1995 amending Council Directive 91/414/EEC: Annex II: 7.2.1.3.2 Water/Sediment Study

Richtlinie für die Prüfung von Pflanzenschutzmitteln, Teil IV, 5-1, Abbaubarkeit und Verbleib von Pflanzenschutzmitteln im Wasser/Sediment System. Dezember 1990. BBA Deutschland, Dezember 1990.

OECD Guidelines for Testing of Chemicals, Aerobic-Anaerobic Transformation in Water-Sediment Systems, Draft Document, November 1998.

GLP: yes (Novartis Crop Protection AG, GLP Quality Assurance, Product Safety Services, CH - 4002 Basel, Switzerland).

Test system: In the present study, the distribution, degradation and metabolism of phenyl-^{14}C-labelled thiabendazole (specific activity: 2.12 MBq/mg, radiochemical purity: [redacted] in equilibrated water-sediment systems were investigated. The incubation was performed at 20°C in the dark.

The water-sediment systems from a river (Rhine at Möhlin, CH) and from a pond (Froschweiher at Rheinfelden, CH) consisted of filtered natural water and the uppermost 5 to 10 cm of sediment sieved through a 2 mm mesh. The test systems (1 litre all-glass metabolism flasks with inner diameter of ca 10 cm and a surface area of ca 78.5 cm^2) were acclimated under laboratory conditions (20 ± 2°C, in the dark) for about 4 weeks until constant levels of pH, oxygen concentration and redox potential were achieved. A water depth of ca 6 cm and a sediment layer of ca 2.5 cm were used in the systems. The water volume added to the test vessels was 500 ml for both systems and sediment corresponded to about 254 g dry sediment or 438 g wet sediment for the river system and to ca 184 g dry sediment or 402 g wet sediment for the pond system. Ventilation of the test systems with

moistened air was at a flow rate of ca 60 ml/min and gentle agitation of the water surface was achieved by means of a magnetic stirrer without disturbing the sediment. The outcoming air was passed through a volatile trapping system consisting of ethylene glycol, one bottle of 0.1N H_2SO_4 and two bottles of 2N NaOH (50 ml each). One month after application the trapping flasks containing ethylene glycol and 0.1N H_2SO_4 were removed due to the low amounts of radioactivity present. The absorption traps were initially exchanged every week, thereafter in one to four weeks intervals. Radioactivity in the NaOH traps was checked for carbon dioxide production by $BaCO_3$ precipitation with 1M $BaCl_2$.

Per aquatic system, 18 samples treated at a normal dose corresponding to ca 0.12 kg ai/ha and 5 samples treated at a high dose of 0.9 kg ai/ha (to gain sufficient amounts of potential metabolites for identification purposes if needed) were set up. In addition, per system several untreated flasks were set up for biomass determinations and follow-up of the maintenance of equilibrium conditions.

A total amount of approximately 93 and 707 µg of the labelled test substance was applied to the water surface resulting in a concentration of 0.186 and 1.414 mg/l water for the low and the high dose treatment, respectively, corresponding to target application rates of about 0.12 and 0.9 kg ai/ha, respectively. Samples were incubated for up to 181 days. The low dose flasks were sampled for analysis after 0, 1, 4, 14, 30, 42, 62, 98, 127 and 181 days (some of them in duplicates) whereas the high dose flasks were sampled after 62, 98, 127 and 181 days of incubation. Characteristics of the test systems are shown in Table 9.2 – 4.

For work-up the water phase was withdrawn from the sediment by decanting through a filter without disturbing the sediment. Total water radioactivity was determined using LSC (liquid scintillation counting) and the water evaporated. The residue was re-dissolved in acetonitrile:water (1:1, v/v) and submitted to HPLC and 2D-TLC for evaluation of the radioactivity distribution pattern.

Sediments were extracted two times under reflux with acetonitrile/water (9/1, v/v), followed by two harsh extractions over 2 hours each using acetonitrile/0.1 N HCl (9/1, v/v) since extraction yields with conventional mild extractions were poor. Subsequently, a final Soxhlet extraction was performed with acetonitrile. Extract radioactivity was measured each time by LSC. Evaporation of the organic solvent, partitioning of the water phase three times into methylene chloride and concentration of the organic phase preceded the analysis via HPLC and TLC. Each extracted sediment residue was eventually combusted.

Additional harsh alkaline extractions of the extracted sediment ('organic matter fractionation') following Soxhlet were performed with selected samples (days 62 and 181) using 0.5N NaOH. The insoluble humin fraction was precipitated and analysed for radiocarbon by combustion. The soluble fraction containing fulvic and humic acids was partitioned with methylene chloride, the organic solvent concentrated and submitted to analysis. Radioactivity distribution and recovery in the fractions was measured by LSC, TLC or HPLC with the final combustion of extracted sediment samples.

: *Water/Sediment Characteristics of River and Pond Systems at Start of Experiments (Ulbrich 1999)*

Sediment characteristics:	**River**	**Pond**
sand [%]	42.08	25.68
silt [%]	45.83	54.42
clay [%]	12.09	19.90
pH	7.3	7.07
Total nitrogen [%]	0.14	0.22
Total phosphorous [%]	0.056	0.046
Organic carbon [%]	1.71	4.09
CEC [mVal/100g sediment]	11.16	22.55
Biomass [mg C/100g dry sediment]	46.0	56.9
Water characteristics:		
pH	8.4	7.7
Oxygen content, surface [mg/l]	6.2	1.6
TOC [mg C/l]	1.4	4.0
Carbonate [mg/l]	124	213
Total nitrogen [mg/l]	< 5.0	< 5.0
Total phosphorous [%]	< 0.1	< 0.1
Hardness [°dH]	7.78	11.9
Redoxpotential [mV]	85	40

Degradation or dissipation rates and corresponding half-lives of thiabendazole were calculated assuming pseudo first order one- or two-compartment (bi-phasic) regression kinetics with ORIGIN software (Microcal Software Inc., Northampton, MA, USA). For the one-compartment model, DT_{50} and DT_{90} values were obtained according to DT_{50} = ln 2/k and DT_{90} = ln 10/k, whereas for the two-compartment model besides the individual parameters for each compartment the overall parameters (comprising the data of both compartments) were calculated by iteration using the Microsoft Excel Solver function.

Findings: The physicochemical characteristics of the test systems during the 181 day incubation period were (mean values over all sampling points): pH 8.1, temperature 20.1°C, oxygen 6.4 mg/l, redox potential +205 mV (water) and –214 mV (sediment) for the river system and pH 8.3, temperature 20.1°C, oxygen 6.4 mg/l, redox potential +209 mV (water) and –208 mV (sediment) for the pond system.

The distribution and nature of radioactivity for the whole incubation period in the river and pond water/sediment systems for the low dose applications are shown in Tables 9.2 – 5 and 9.2 – 6, respectively.

The radioactivity recovered averaged 100.3 ± 2.9% and 97.8 ± 3.3% of the totally applied low dose for the river and pond system, respectively, and 97.6 ± 2.0 % and 94.9 ± 1.8 % of the applied high dose, respectively.

In both systems the total radioactivity in the water phase decreased rapidly over the incubation period: in the river water it decreased from 96.5% at day 0 to 2.1% of the applied low dose at day 14 remaining at levels below 0.7 % till study end, and in the pond water it declined from 92.2% at day 0 to 7.0% of the applied low dose at day 14 remaining at levels below 0.7 % till study end. Radioactivity in the high dose water samples was always less than 0.6% of applied dose (certainly due to the fact that the first sampling after treatment was done at day 62 only).

In the river sediment, non-extractable radioactivity increased continuously to a level of 23.1% till study day 14 and varied thereafter on a rather constant level between 32.3 and 25.7% of applied low dose till study termination. The high dose sample at day 181 accounted for 26.0% of applied dose. Extractable radioactivity increased to a maximum of 76.1% of applied low dose after 42 days (high dose: 80.3% at day 62) and decreased slightly towards study termination to 70.9% (high dose: to 72%). Alkaline extractions with 0.5N NaOH at days 62 and 181 released additional 26.1% and 17.1% of applied low dose, respectively, 9.5% and 10.7% thereof being associated with the insoluble humin fraction.

All volatile radioactivity in the river system was characterised as carbon dioxide. It reached 0.5% and 0.7% at day 181 for the low and high dose samples, respectively, indicating little mineralisation.

In the pond sediment, non-extractable radioactivity increased continuously within 42 days to a level of 65.2 % till study day 30 and varied thereafter on a rather constant level between 49.1 and 65.4% of applied low dose till study termination. The high dose sample at day 181 accounted for 61.6% of applied dose. Extractable radioactivity increased to a maximum of 44.3% of applied low dose after 42 days (high dose: 48.7% at day 62) and decreased towards study termination to 29.3% (high dose: to 30.5%). Alkaline extractions with 0.5N NaOH at days 62 and 181 released additional 35.3% and 36.2% of applied low dose, respectively, 25.2% and 27.5% thereof being associated with the insoluble humin fraction.

All volatile radioactivity in the pond system was characterised as carbon dioxide. It reached 1.8% and 1.2% at day 181 for the low and high dose samples, respectively, indicating little mineralisation.

Parent thiabendazole disappeared rapidly from the water phases of both systems: in the river water it dropped from initially 96.6% of applied low dose to 22.3% at day 4. Since at day 14 the total radioactivity in water accounted for 2.1% only, the thiabendazole concentration must have been even less. Therefore, the river water was not further analysed for thiabendazole as of day 14 (since predicted to be < 2.1% of applied dose). Thiabendazole in the high dose samples was not analysed at all due to the late first sampling at day 62 (predicted to be less than 0.6% of the applied dose). No degradation products were detected in the river water.

Similarly to the river system thiabendazole disappeared rapidly from the pond water phase: it dropped from initially 92.2% of applied low dose to 6.3% at day 14. Since at the further sampling intervals the total radioactivity in pond water never exceeded 0.7% till study end, the thiabendazole concentration must have been even less at these study days. Therefore, the pond water was not further analysed for thiabendazole as of day 30 (since predicted to be < 0.7% of applied dose). Thiabendazole in the high dose samples was not analysed at all due to the late first sampling at day 62. No metabolites were detected as well in the pond water.

The concentration of thiabendazole in the river sediment increased to a maximum of 76.1% of the applied low dose radioactivity at day 42. Towards study end, the concentration slightly decreased to 70.9%. Besides the parent substance, one unknown metabolic fraction was observed in the extracts not exceeding 1.6% (day 98) of

the applied radioactivity. The high dose samples did not exhibit significant differences with respect to the distribution pattern and dissipation behaviour of thiabendazole.

The concentration of thiabendazole in the pond sediment increased to a maximum of 44.3% of the applied low dose radioactivity at day 42. Towards study end, the concentration decreased to 29.3%. One unknown metabolic fraction was observed in the extracts not exceeding 1.2% (day 62) of the applied radioactivity. The high dose samples did not exhibit significant differences with respect to the distribution pattern and dissipation behaviour of thiabendazole.

The radioactivity measured in the soluble fractions after organic matter fractionation (alkaline treatments of extracted sediment samples at days 62 and 181) and partitioning to methylene chloride was shown to consist of thiabendazole only with maximum amounts of 26.1% (river, day 62) and 36.2% of applied low dose (pond, day 181).

*Radioactivity Distribution of Phenyl-^{14}C-Labelled Thiabendazole in **River Aquatic System** as Percent of the Applied Low Dose Presenting Mean Values where Available (Ulbrich 1999)*

Time [days]	Water Layer [%]	Sediment Extractables [%]	Total System [%]			Non Extractables [%]	CO_2 [%]	Total Recovery [%]
	thiabendazole	thiabendazole	thiabendazole	fraction M1	unresolved			
0	96.6	n.a.*	96.6	<LD**	<LD	1.8	n.a.*	98.4
1	53.9	38.8	92.7	0.9	<LD	7.7	<0.1	101.3
4	22.3	64.4	86.7	<LD	<LD	12.6	<0.1	99.3
14	n.a.*	74.3	74.3	<LD	2.1	23.1	0.1	99.7
30	n.a.	73.5	73.5	<LD	0.7	28.4	0.2	102.7
42	n.a.	76.1	76.1	<LD	0.3	18.4	0.4	95.2
62	n.a.	70.3	70.3	0.9	0.3	32.3	0.4	104.4
98	n.a.	69.7	69.7	1.6	0.3	30.2	0.3	102.1
127	n.a.	74.9	74.9	0.6	0.5	24.0	0.4	100.3
181	n.a.	70.9	70.9	<LD	0.2	25.7	0.5	97.2

* n.a. = not analysed; ** LD = limit of detection

*Radioactivity Distribution of Phenyl-^{14}C-Labelled Thiabendazole in **Pond Aquatic System** as Percent of the Applied Low Dose Presenting Mean Values where Available (Ulbrich 1999)*

Time [days]	Water Layer [%]	Sediment Extractables [%]	Total System [%]			Non Extractables [%]	CO_2 [%]	Total Recovery [%]
	thiabendazole	thiabendazole	thiabendazole	fraction M1	unresolved			
0	92.2	n.a.*	92.2	<LD**	<LD	1.1	n.a.*	93.4
1	63.4	19.1	82.5	<LD	<LD	15.2	<0.1	97.7
4	28.8	20.1	48.9	<LD	<LD	47.9	<0.1	96.8
14	6.3	37.5	43.8	<LD	<LD	52.7	0.1	97.2
30	n.a*.	33.1	33.1	<LD	0.3	65.2	0.4	98.9
42	n.a.	44.3	44.3	<LD	0.3	49.1	0.2	94.0
62	n.a.	43.2	43.2	0.6	0.3	55.5	0.5	100.1
98	n.a.	38.1	38.1	<LD	0.3	60.8	0.7	99.9
127	n.a.	39.7	39.7	<LD	0.7	59.7	0.9	101.0
181	n.a.	29.3	29.3	<LD	0.5	65.4	1.8	97.1

* n.a. = not analysed; ** LD = limit of detection

The dissipation rates of thiabendazole from the aqueous phases were calculated by applying pseudo first order one-compartment reaction kinetics. The results are shown in Table 9.2 – 7. Thiabendazole disappeared from the water phase with a DT_{50} of 1.6 days for the river and of 2.3 days for the pond system with the coefficient of determination, $R^2 > 0.98$. The corresponding DT_{90} values were 5.3 and 7.8 days, respectively.

Dissipation Times of Thiabendazole in Aquatic Systems (Ulbrich 1999)

	Water (one compartment kinetics) [days]		**Total System (two compartment kinetics) [days]**	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}
River	1.6	5.3	4.3 (1st comp., =14 d)	-
Pond	2.3	7.8	4.4* (1st comp., =14 d)	-
River (2nd comp., = 14d; extrapolated)	-	-	4332	-
Pond (2nd comp., = 14d; extrapolated)	-	-	375	-
River (iteration and extrapolation)	-	-	overall: 2406	12465
Pond (iteration and extrapolation)	-	-	overall: 5.4	825

* This value was re-evaluated, since the report provides a DT_{50} of 1.6 days, which was obviously due to an erroneous calculation

The dissipation of thiabendazole from the total systems was characterised by a bi-phasic decrease, hence a first order two-compartment model was applied. The results are presented also in Table 9.2 – 7 above.

In the first phase, a rapid partitioning of the active substance to the sediment followed by strong binding was observed within the first 14 days, resulting in whole system thiabendazole DT_{50} values of 4.3 days and 4.4 days for river and pond, respectively, and in relatively large amounts of non-extractable residues depending on the sediment properties (organic matter, clay, cation exchange capacity) in rather short time periods. In the pond system, the amount of strongly bound radioactivity was about twice as high as in the river system reflecting the higher sorptive capacity of this sediment.

In the second phase the decline of thiabendazole was quite slow as demonstrated by whole system DT_{50} values of > 4000 days and 375 days for the river and the pond system, respectively.

The Excel solver function, iterating the degradation data over the whole study period, provided finally overall whole system DT_{50} values of 2406 and 5.4 days for the river and the pond system, respectively. However, due to the slow decline in the second phase, the corresponding overall DT_{90} values are not closely related to the overall DT_{50} values (by a factor of 3.32 as it is the case for nonlinear one-compartment first-order kinetics), as demonstrated by the high DT_{90} values of 12465 to 875 days calculated, based on very large and hence very uncertain extrapolations.

Overall, the degradation rates reported are considered to be conservative values, since the harsh extraction procedures applied (2x acidic reflux) cannot be regarded to be representative for naturally occurring thiabendazole desorption processes; therefore, the actual amount of the non-extractable sediment fraction is expected to be higher and the actual thiabendazole sediment concentration to be lower under field conditions in such sediments as used in this study.

In conclusion, the negligible mineralisation rate, i.e. CO_2 formation, the poor pattern of metabolites and the fact that with sodium hydroxide (organic matter fractionation) considerable amounts of the parent compound can be released from the bound residue fraction, suggest that thiabendazole mainly disappears from aquatic systems by physico-chemical processes and not by microbial degradation.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09-27-2004
Reference	Ulbrich, R. (1999) MK 360 (CGA 28020): Degradation and Metabolism of ^{14}C-Phenyl-Labelled MK 360 in Two Aerobic Aquatic Systems under Laboratory Conditions.

Document IIIA_sec 7b submitted by applicant: 7.1.2 / 02.

Document IVA_sec submitted by applicant: 7.1.2.2.1.

Materials and Methods

[redacted]

Results and discussion

[redacted]

[redacted]

Conclusion

[redacted]

	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	**7.1.2 / 01**	**Rate and route of degradation in aquatic systems including identification of metabolites and degradation prodcuts**
91/414 Annex Point addressed	**II 7.1.1.1.2 / 01**	**Supplementary soil degradation studies - anaerobic degradation**

1.2	**Title**	Anaerobic Soil Metabolism of [^{14}C] Thiabendazole
1.3	**Report No.**	37640
1.4	**Lab. report No.**	not applicable
1.5	**Cross reference**	7.1.1.2.1/01
1.6	**Authors**	Donna Daly - Supervisor, Environmental Fate
1.7	**Date of report**	26 June 1990
1.8	**Published**	no
2.1	**Testing facility**	Analytical Bio-Chemistry Laboratories, Inc., P.O. Box 1097, Columbia, MO 65205, USA
2.2	**Dates of experimental work**	22 March 1989 to 3 November 1989
3	**Objective**	to establish the pattern of anaerobic metabolism and degradation of Thiabendazole in the soil
4.1	**Test substance**	Thiabendazole [2-(4-thiazolyl)-1H-benzimidazole], phenyl-U-^{14}C Composition: uniformly labelled with ^{14}C in the phenyl ring with a specific activity of 24.77 microcuries/mg and radiochemical purity of [redacted] ^{14}C]Thiabendazole
4.2	**Specification**	[redacted]
4.4	**Stability in vehicle**	stable
4.5	**Homogeneity in vehicle**	homogenous
4.6	**Validity**	not applicable
5	**Vehicle/solvent**	methanol
6	**Physical form**	powder
7.1	**Test method**	Study design follows EPA/FIFRA subdivision N guidelines, 162-2, 1982
7.2	**Justification**	not applicable
7.3	**Copy of method**	description of methods included in report
8	**Choice of method**	not applicable
9	**Deviations**	not applicable
10.1	**Certified laboratory**	not applicable
10.2	**Certifying authority**	not applicable
10.3	**GLP**	yes
10.4	**Justification**	not applicable
11.1	**GEP**	not applicable
11.2	**Type of facility (official or officially**	

	recognized)	not applicable
11.3	**Justification**	not applicable
12	**Test system**	
	Test soil:	sandy loam
	Test conditions:	ten 1 g samples of soil were placed in 50-ml Pyrex culture tubes at a moisture content of 35% of field capacity
	Duration of test:	three months
	Applied rate:	study soil was treated with sufficient Thiabendazole to achieve a nominal concentration of 1.04 µg/g. This is equivalent to the maximum commercial rate.
	Temperature:	the study was conducted in an environmental chamber regulated at 25 ±1°C.
	Aerobic/anaerobic:	following a 1-month aerobic aging period, the test soils were flooded with water, purged with nitrogen gas, and allowed to age under anaerobic conditions for an additional 60 days.
	Sampling:	samples were collected on 0, 1, 3, 7, 14 days and 1 month after aerobic initiation, and at 15, 30, 45 and 60 days after anaerobic initiation. Microbial viability of the test soil was assayed before and after the aerobic phase and again after the anaerobic phase. The samples for chemical and radio analysis were exhaustively extracted first with 1N methanolic KOH and then with 6N HCl:DMF (1:1 v:v)
	Analytical methods:	soil samples were extracted and analyzed by high pressure liquid chromatographic analysis (HPLC). Measurements of radioactivity were made using liquid scintillation counting. The amounts of non-extractable (bound) residues were determined at 5, 7, 8, 10 and 11 months by combustion of the post-extracted soil and analysis of the trapped combustion products by LSC. The radiochemical purity of the ^{14}C-Thiabendazole was determined by reversed phase thin-layer chromatography (TLC).

13 Findings

Distribution of ^{14}C-residues of Thiabendazole during the aerobic soil metabolism study (percent of dose)[a]										
							After establishing anaerobic conditions			
	Day 0	Day 1	Day 3	Day 7	Day 14	1 Mon.	Day 15	Day 30	Day 45	Day 60
Total ^{14}C-Accountability[b]	100.0	98.8	95.3	99.8	102.9	98.1	99.4	100.7	99.4	100.3
Cumulative volatiles	0.0000	0.0114	0.0468	0.0922	0.170	0.677	0.768	0.791	0.799	0.820
Ethylene glycol	0.0000	0.0030	0.0091	0.0173	0.0247	0.0540	0.0718	0.0718	0.0763	0.0763
H_2SO_4	0.0000	0.0048	0.0048	0.0111	0.0121	0.0146	0.0181	0.0181	0.0181	0.0212
KOH1 + KOH2	0.0000	0.0036	0.0328	0.0638	0.1330	0.608	0.679	0.701	0.705	0.722
Water soluble residues	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.43	0.40	0.47
Non-extractable residues	0.62	2.0	3.7	2.4	3.5	5.8	5.6	5.7	6.2	5.5
Extractable residues	99.4	96.8	91.6	97.3	99.2	91.6	92.6	93.7	92.0	93.5
Soil extract I	92.5	79.8	70.2	76.2	75.2	59.0	63.0	63.8	64.4	65.9
characterized as Thiabendazole	83.9	71.3	67.5	71.7	69.4	53.3	59.2	60.8	60.8	62.6
characterized as Benzimidazole	5.4	4.7	0.4	1.4	1.0	2.2	0.3	0.6	0.7	0.5
Soil extract II	6.8	17.0	21.4	21.1	24.1	32.6	29.6	29.9	27.5	27.6
characterized as Thiabendazole	4.3	8.6	12.0	12.3	17.8	20.8	15.4	17.0	16.0	15.4
characterized as Benzimidazole	2.4	9.1	11.8	9.3	6.5	6.1	7.7	5.7	5.0	4.9

a Initial measure dose = 1.04 µg Thiabendazole equivalents/g of soil
b Mean ^{14}C-accountability = 99.5% ±2.0%

NOTE: all figures are based on the mean of duplicate samples

	Results:	Based on the data generated in this 90-day anaerobic soil metabolism study conducted under dark conditions, the half life for [^{14}C]Thiabendazole was calculated to be 211days for aerobic aging. [^{14}C]Thiabendazole appeared to be stable under anaerobic conditions, based on the results of the HPLC analysis. At the end of the 90-day study, the only degradation product identified was benzimidazole, which accounted for 5.4% of the initial measured dose compared to 8.3% present after the initial aerobic phase. Microbial viability was verified at the start and finish of the aerobic phase, as well as after the anaerobic phase of the trial. The mean ^{14}C-mass balance was 99.5 ±2.0% of the IMD (initial measured dose).
14	**Statistics**	no statistical analysis was carried out, as it was considered unnecessary for interpretation of the results and therefore not required
15	**References to publications**	none
16	**Unpublished data**	not applicable

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04-07-2005
Refence	Daly, D. (1990a) Anaerobic Soil Metabolism of [^{14}C]-Thiabendazole. Document IIIA_sec 7b submitted by the applicant: 7.1.2 / 03. Document IVA_sec submitted by the applicant: 7.1.2.1.
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

98/8 Doc IIIA section No.	**7.1.3 / 02**	**Adsorption studies screeing test**

Studies presented later under 7.2.3.1.

7.2Fate and Behaviour in soil

98/8 Doc IIIA section No.	**7.2.1 / 01**	**Aerobic degradation on soil, initial studies**
91/414 Annex Point addressed	**II 7.1.1.1.1 / 01**	**Soil route of degradation: Aerobic degradation**

1.2	**Title**	Aerobic Soil Metabolism of [^{14}C] Thiabendazole
1.3	**Report No.**	37639
1.4	**Lab. report No.**	not applicable
1.5	**Cross reference**	7.1.1.2.1/02
1.6	**Authors**	Donna Daly - Supervisor, Environmental Fate
1.7	**Date of report**	24 January 1991
1.8	**Published**	no
2.1	**Testing facility**	Analytical Bio-Chemistry Laboratories, Inc., P.O. Box 1097, Columbia, MO 65205, USA
2.2	**Dates of experimental work**	30 March 1989 to 23 August 1990
3	**Objective**	to establish the pattern of aerobic metabolism and degradation of Thiabendazole in the soil
4.1	**Test substance**	Thiabendazole [2-(4-thiazolyl)-1H-benzimidazole], phenyl-U-^{14}C Composition: uniformly labelled with ^{14}C in the phenyl ring with a specific activity of 24.77 microcuries/mg and radiochemical purity of [redacted][^{14}C]Thiabendazole
4.2	**Specification**	[redacted]
4.4	**Stability in vehicle**	stable
4.5	**Homogeneity in vehicle**	homogenous
4.6	**Validity**	not applicable
5	**Vehicle/solvent**	methanol
6	**Physical form**	powder
7.1	**Test method**	Study design follows EPA/FIFRA subdivision N guidelines, 162-1, 1982
7.2	**Justification**	not applicable
7.3	**Copy of method**	description of methods included in report
8	**Choice of method**	not applicable
9	**Deviations**	not applicable

10.1	**Certified laboratory**	not applicable
10.2	**Certifying authority**	not applicable
10.3	**GLP**	yes
10.4	**Justification**	not applicable
11.1	**GEP**	not applicable
11.2	**Type of facility (official or officially recognized)**	not applicable
11.3	**Justification**	not applicable

12 Test system

Test soil: microbially active sandy loam

Test conditions: under dark conditions, ten 1 g samples of soil were placed in 50-ml Pyrex culture tubes at a moisture content of 35% of field capacity

Duration of test: one year

Applied rate: study soil was treated with sufficient Thiabendazole to achieve a nominal concentration of 1 µg/g. This is equivalent to the maximum commercial rate. The concentration of Thiabendazole in the test system immediately after dosing was determined to be 1.05 µg/g.

Temperature: the study was conducted in an environmental chamber regulated at 25 ±1°C.

Moisture: soil moisture levels were monitored on prescribed days and adjusted after treatment to maintain a nominal moisture level of approximately 70-75% of field capacity

Sampling: duplicate soil samples were collected on 0, 1, 3, 7, 14 days and 1, 2, 3, 4, 6, 9 and 12 months following dosing.

Microbial viability of the test soil was assayed at 3-month intervals during the trial. The samples for chemical and radio analysis were exhaustively extracted first with 1N methanolic KOH and then with 6N HCl:DMF (1:1 v:v)

Analytical methods: soil samples were extracted and analyzed by high pressure liquid chromatographic analysis (HPLC).

Measurements of radioactivity were made using liquid scintillation counting.

The amounts of non-extractable (bound) residues were determined at 5, 7, 8, 10 and 11 months by combustion of the post-extracted soil and analysis of the trapped combustion products by LSC.

The radiochemical purity of the ^{14}C-Thiabendazole was determined by reversed phase thin-layer chromatography (TLC).

13 Findings	Distribution of ^{14}C-residues of Thiabendazole during the aerobic soil metabolism study (percent of dose)[a]											
	Day 0	Day 1	Day 3	Day 7	Day14	1 Mon.	2Mon.	3Mon.	4Mon.	6Mon.	9Mon.	12Mon
Total ^{14}C-Accountability[b]	100.0	95.0	97.1	98.0	101.7	94.1	95.8	97.0	91.5	94.4	99.5	92.0
Cumulative volatiles	0.000	0.009	0.026	0.070	0.106	0.749	1.78	2.94	3.88	4.69	5.25	5.83
Ethylene glycol	0.000	0.000	0.000	0.011	0.011	0.0113	0.011	0.011	0.042	0.063	0.156	0.2257
H_2SO_4	0.000	0.009	0.009	0.009	0.009	0.0099	0.009	0.009	0.016	0.016	0.016	0.0167
KOH1 + KOH2	0.000	0.000	0.016	0.049	0.085	0.728	1.75	2.92	3.82	4.61	5.08	5.59
Non-extractable residues	1.24	1.6	3.3	2.8	2.9	6.3	13.9	16.2	20.2	15.6	14.7	12.5
Extractable residues	98.8	93.4	93.8	95.2	98.6	87.0	80.1	77.8	67.4	74.1	79.5	73.6
Soil extract I (1N methanolic KOH)	89.8	78.1	66.8	74.3	75.1	61.3	56.8	56.5	38.8	40.2	33.8	32.5
characterized as Thiabendazole	82.4	59.8	59.9	48.4	64.2	50.4	46.0	49.2	35.5	34.6	30.7	29.4
characterized as Benzimidazole	0.00	2.20	0.39	1.80	0.00	0.97	0.78	0.77	0.21	0.31	0.38	0.17
characterized as 5-OH-Thiabendazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
characterized as Unknown 1	0.95	7.40	3.71	11.94	7.25	2.44	5.25	2.22	0.53	2.19	0.93	0.86
characterized as Unknown 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.00
Soil extract II (1:1 6N HCl:DMF)	9.0	15.3	26.9	20.9	23.6	25.7	23.3	21.3	28.6	33.9	45.7	41.2
characterized as Thiabendazole	6.7	11.9	22.5	17.1	21.2	22.8	18.3	14.5	20.7	23.9	30.7	27.4
characterized as Benzimidazole	0.00	0.00	0.16	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
characterized as 5-OH-Thiabendazole	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.25	0.27	0.00	0.00
characterized as Unknown 1	0.00	0.17	0.29	0.35	0.25	0.39	0.00	0.00	0.00	0.00	0.00	0.00
characterized as Unknown 2	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a Initial measure dose = 1.05 µg Thiabendazole equivalents/g of soil

b Mean 14C-accountability = 96.3% ±3.3%

NOTE: all figures are based on the mean of duplicate samples

	Results:	Based on the data generated in this one-year aerobic soil metabolism study conducted under dark conditions, the half life for [^{14}C]Thiabendazole was calculated to be 1.5 to 2 years. At the end of the year-long study, extractable ^{14}C-residues accounted for 73.6% of the initial measured dose (IMD), non-extractable ^{14}C-residues amounted to 12.5% of IMD and the cumulative ^{14}C-volatile residues accounted for 5.83% of the IMD. The majority (5.59% of the IMD) of the volatile residue was shown to be $^{14}CO_2$. At the end of the study, the degradation product benzimidazole was identified, and accounted for 2.2% of the initial measured dose at day 1 and 0.17% at the termination of the study. Traces of 5-OH-TBZ were also found. Microbial viability was verified every three months for the duration of the trial. The mean ^{14}C-mass balance at the end of the trial was 96.3% of the IMD.
14	**Statistics**	no statistical analysis was carried out, as it was considered unnecessary for interpretation of the results and therefore not required
15	**References to publications**	none
16	**Unpublished data**	not applicable

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04-06-2005
Reference	Daly, D. (1991b) Aerobic Soil Metabolism of [^{14}C]-Thiabendazole. Document IIIA_sec 7b submitted by the applicant: 7.2.1 / 01 Document IVA_sec submitted by the applicant: 7.2.1.
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**7.2.1 / 02**	**Aerobic degradation on soil, initial studies**
91/414 Annex Point addressed	**II 7.1.1.1.1 / 01**	**Soil route of degradation: Aerobic degradation**

1.2	**Title**	Rate of Degradation of ^{14}C-Phenyl-Labelled MK 360 in one Soil under Various Laboratory Conditions at 20°C and 30°C
1.3	**Report No.**	98RP06
1.5	**Cross reference** 7.1.1.2	
1.6	**Authors**	Phaff, R
1.7	**Date of report**	13 December 1999
1.8	**Published**	no
2.1	**Testing facility**	Novartis Crop Protection AG, Basel, Switzerland
3	**Objective**	to establish the rate of degradation of ^{14}C-Phenyl-Labelled MK 360 in the soil

Report: Laboratory Soil Degradation of Thiabendazole

Analytical methods:

Report: Phaff, R.. (1999): Rate of Degradation of ^{14}C-Phenyl-Labelled MK 360 in one Soil under Various Laboratory Conditions at 20°C and 30°C. Novartis Crop Protection AG, Environmental Safety, Ecochemistry, CH – 4002 Basel, Switzerland. Unpublished report, Proj.No 98RP06, 13 December 1999.

Guideline: Commission Directive 95/36/EC amending Council Directive 91/414/EEC: Annex II: 7.1 Fate and Behaviour in Soil, 7.1.1.2 Rate of Degradation.

Additionally the following guidelines were taken into account:

Richtlinie für die amtliche Prüfung von Pflanzenschutzmitteln, Teil IV, 4-1: "Verbleib von Pflanzenschutzmitteln im Boden: Abbau, Umwandlung und Metabolismus". Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland, Dezember 1986.

Dutch Registration Guideline, Section G.1: Behaviour in Soil; Ministry of Agriculture and Fisheries, Ministry of Public Health and Environmental Hygiene, Ministry of Social Affairs, January 1987.

GLP: yes (Novartis Crop Protection AG, GLP Quality Assurance, Product Safety Services, CH – 4002 Basel, Switzerland)

Test system: In the present soil degradation study the influence of soil moisture, soil temperature and the initial active substance concentration on the degradation rate of thiabendazole was investigated over a study period of 120 days in four subprojects. Soil aliquots of 75 g dry soil equivalents (2 mm sieved Gartenacker silt loam soil) in 300 ml Erlenmeyer flasks were dosed with phenyl-U-^{14}C-labelled thiabendazole (specific activity: 2.12 MBq/mg; radiochemical purity: 100.0%) at target concentrations of 0.1 or 1.0 mg ai/kg corresponding to field rates of approximately 100 and 1000 g ai/ha, respectively, assuming a penetration depth of 10 cm and a soil bulk density of 1.0 g/cm^3. The soil characteristics are given below

Characteristics of Gartenacker soil used for thiabendazole degradation studies (Phaff 1999)

Test material used in study	Phenyl-^{14}C-labelled thiabendazole

Sample ID		Gartenacker soil, batch 7/98
Classification (USDA)		silt loam
pH (KCl)		7.3
Organic carbon [%]		2.11
CEC [meq/100 g soil]		14.3
Maximum water holding capacity (MWC; pF < 0.3), [g H_2O/100g dry soil]		63.7
Field capacity (FC; pF = 2.5) [g H_2O/100g dry soil]		48.0
Bulk density [g/cm^3]		1.14
Total nitrogen [%]		0.23
Particle size:	Clay [%]	7.21
	Silt [%]	50.37
	Sand [%]	42.42
Microbial biomass	[mg C/ 100 g soil]	52 (day 0) 37 (day 120)

The treated soils were incubated in the dark under the following conditions at an air-flow rate of ca 25 ml/min: 40% MWC at 20°C at a concentration of 1.0 mg/kg (test 1), 25% MWC at 20°C (test 2), 40% MWC at 20°C (test 3) and 40% MWC at 30°C (test 4). Tests 2, 3 and 4 were performed at a concentration of 0.1 mg/kg thiabendazole. Volatiles were trapped in ethylene glycol and two times 2N NaOH and analysed for radioactivity at one- to bi-weekly intervals. Presence of carbon dioxide was proven e.g. with $BaCO_3$ precipitation for selected samples. Soil samples were taken at days 0, 8, 14, 28, 56, 90 and 120 and submitted to exhaustive extraction using acetonitrile:water 80:20 (v/v) several times followed by a final Soxhlet extraction with acetonitrile for 6 hours. Soxhlet extracts containing less than 5% of applied dose were combined with the room temperature extracts and otherwise processed separately. The extracts were analysed and profiled by LSC, HPLC and 2D-TLC (as the confirmatory method) and a radioactivity balance and distribution of radioactivity was established for each sample. Residual radiocarbon in the extracted soil samples was determined via combustion.

As of day 8, Soxhlet extracted soil samples were in addition subjected to harsh neutral and acidic reflux extractions using acetonitrile:water 80:20 (v/v) and acetonitrile:0.1M HCl at 80°C for 2 hours. An organic matter fractionation was furthermore applied to the test 3 soil sample of day 120 by treatment with 0.5N NaOH. This provided the insoluble humin fraction and a soluble fraction which was separated by addition of concentrated HCl yielding the precipitated humic acid and the soluble fulvic acid fraction.

Biomass was determined according to the glucose induced short-term respiration method.

The rates of degradation of thiabendazole in soil were calculated applying pseudo first order two-compartment (bi-phasic) regression kinetics with ORIGIN software (Microcal Software Inc., Northampton, MA, USA). DT_{50} and DT_{90} values for the two-compartment model over the whole study period and beyond were calculated by iteration using the Microsoft Excel Solver function.

Findings:

The degradation pattern and the overall recovery of the phenyl-labelled test substance under various conditions in the silt loam soil are shown in the table. The recoveries ranged from 89.3 % to 99.3 % of the dose applied for all conditions. The total extractable radioactivity (sum of extraction at room temperature, Soxhlet extraction, and harsh extraction) decreased during the study between days 0 to 120 from 89.9% to 44.8% of applied dose (Test 1), from 93.5% to 52.5% (Test 2), from 94.6% to 27.2% (Test 3) and from 90.0% to 21.2% of applied dose (Test 4). The extractables at room temperature decreased from between 88.7 and 84.2 % at study start to between 5.2 and 23.5 % of the applied radioactivity at study end. Soxhlet extractions recovered at highest between 10.8 and 12.1 % of the applied radioactivity. The neutral and acidic reflux treatments (harsh extractions) extracted up to a maximum of 4 % of the applied dose till incubation day 28. Thereafter, till study end, 15.1 to 27.5 %. were extractable by this procedure with maximum contributions of between 20.9 and 27.5% of the applied dose depending on the test conditions and sampling day.

The non-extractable radioactivity increased to values of 40.2% (20 °C, 40% MWC, 1 mg/kg), 37.9% (20 °C, 25% MWC, 0.1 mg/kg), 56.0% (20 °C, 40% MWC, 0.1 mg/kg) and 55.9% (30 °C, 40% MWC, 0.1 mg/kg) at study end.