Competent Authority Report

According to Directive 98/8/EC on Biocidal Products

Tebuconazole

CAS No.: 107534-96-3

ELINCS No.: 403-640-2

Document III-A.1

from Lanxess Deutschland GmbH

for use in wood preservatives (Product type 8)

Reporting Member State: Denmark

May 2007

Section A1 Applicant

Annex Point IIA1

1.1	**Applicant**	Name: Contact person: Address:	LANXESS Deutschland GmbH [redacted] D-51369 Leverkusen Germany
		Telephone number: Fax number: E-mail address:	0049 214 30 [redacted] 0049 214 30 [redacted] [redacted]
1.2	**Manufacturer of Active Substance** (if different)	Name of plant: Street: Town: Country:	[redacted]
1.3	**Manufacturer of Product(s) (if different)**		Bayer Chemicals AG
	1) Product 1		
	2) Product 2		

Competent Authority Report

According to Directive 98/8/EC on Biocidal Products

Tebuconazole

CAS No.: 107534-96-3

ELINCS No.: 403-640-2

Document III-A.2

Identity

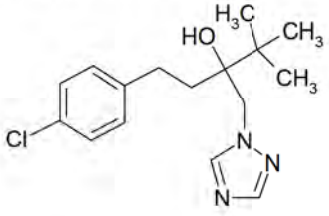
from Lanxess Deutschland GmbH

for use in wood preservatives (Product type 8)

Reporting Member State: Denmark

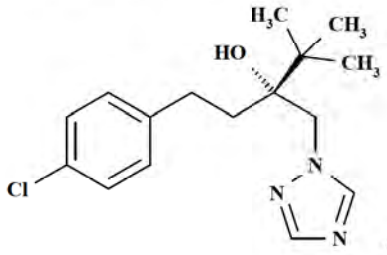
May 2007

Section A2 Identity of Active Substance

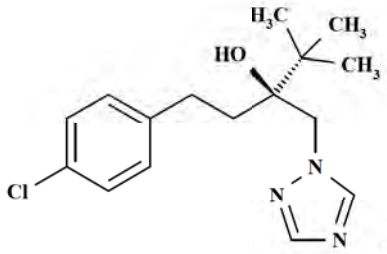
Subsection (Annex point)			Official use only
2.1	**Common name (IIA2.1)**	Common name: Tebuconazole (ISO) Other names: Preventol A 8 (trade name in wood preservation); Preventol VP OC 3047 (trade name in wood preservation during development); Folicur (trade name for spray application); Raxil (trade name for seed dressing); Ethyltrianol, Fenetrazole, Terbutrazole (former proposed, but not accepted common name for HWG 1608 which is used in some reports);	√
2.2	**Chemical name (IIA2.2)**	IUPAC name: (RS)-1-(4--chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-pentan-3-ol; C.A.S. name: 1H-1,2,4-triazole-1-ethanol, .alpha.-[2-(4-chlorophenyl)ethyl]-.alpha.-(1,1-dimethylethyl)-, (.+-)-	√
2.3	**Manufacturer´s development code number(s) (IIA2.3)**	R & D code: HWG 1608, also BAY-HWG 1608	√
2.4	**CAS-No and EC numbers (IIA2.4)**		√
2.4.1	**CAS-No**	107534-96-3	√
	R-enantiomer	120786-56-3	
	S-emantiomer	119364-85-1	
2.4.2	**EC-No**	ELINCS No.: 403-640-2, Index No.: 603-197-00-7	√
	Isomer 1		
	Isomer n		
2.4.3	**Other**	CIPAC No. 494	√
2.5	**Molecular and structural formula, molecular mass (IIA2.5)**		√
2.5.1	**Molecular formula**	C_{16} H_{22} Cl N_3 O	√
2.5.2	**Structural formula**		√
2.5.3	**Molecular mass**	307.8 g/mol	√

Section A2 Identity of Active Substance

2.6	**Method of manufacture of the active substance (IIA2.1)**	cf. CONFIDENTIAL SECTION		√
2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**		According to 5 batch analysis (Baird & Otis 1992) / According to actual specification (Haack, 2005)	√
		Mean Value	[redacted] / -	
		Minimum Value	[redacted] / -	
		Maximum value	[redacted] / > 95 % w/w	
2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	See separate form sheet (Section A2.8, identity of impurities)		√
2.8.1	**Isomeric composition** *Give maximum content of active isomer and ratio isomer/ diastereomers if relevant*	Tebuconazole is a racemic mixture of two enantiomers (1:1):		√



"R-enantiomer" (+)– [120786-56-3]



"S-enantiomer" (–)– [119364-85-1]

2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA2.9)**	n.a.	√

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
EVALUATION BY RAPPORTEUR MEMBER STATE

Section A2 Identity of Active Substance

Date	November 2005
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A2.8 Identity of impurities and additives (active Substance)

Annex point IIA2.8

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A2.10
Annex Point IIA2.10

Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Official use only

Subsection

2.10.1 Human exposure towards active substance

2.10.1.1 Production

i) Description of process

Production of active substance - Part I:
Technical tebuconazole (Preventol A 8) is produced in [redacted]. Therefore a description of the exposure situation during the production process is not necessary.

Production of active substance - Part II ([redacted]):
A minor part of tebuconazole delivered form [redacted] is further processed in the [redacted]. The resulting products Preventol A 8-D and A 8-F both contain 42-47% active as a dispersion in isoparaffins with [redacted] and a slightly different content [redacted].

Tebuconazole technical is delivered in up to 650 kg containing big bags. The big bags are filled [redacted] During this process the technical active [redacted]

ii) Workplace description

Production of active substance - Part I:
Technical tebuconazole (Preventol A 8) is produced in [redacted]. Therefore a workplace description is not necessary.

Production of active substance - Part II ([redacted]):
During the above mentioned processes potential occupational exposure can only occur during the loading/filling processes at the beginning and the end of the process. People involved in these processes wear personal protection including a respirator. During cleaning and service processes personal protection measures will be taken in relation to the kind of the task the expected probability of coming in contact to the active.

iii) Inhalation exposure

Due to the effective personal protective measures during the above mentioned tasks and the closed plant technology including an effective exhaustion neither dermal nor inhalation exposure is expected for the people involved in the production of Preventol A 8-D and A 8-F from technical tebuconazole.

iv) Dermal exposure

Due to the effective personal protective measures during the above mentioned tasks and the closed plant technology including an effective exhaustion neither dermal nor inhalation exposure is expected for the people involved in the production of Preventol A 8-D and A 8-F from technical tebuconazole.

Section A2.10 Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Annex Point IIA2.10

2.10.1.2 Intended use(s)	See Document II-B of dossier.
1. Professional Users	
i) Description of application process	See Document II-B of dossier.
ii) Workplace description	See Document II-B of dossier.
iii) Inhalation exposure	See Document II-B of dossier.
iv) Dermal exposure	See Document II-B of dossier.
2. Non-professional Users including the general public	See Document II-B of dossier.
(i) via inhalational contact	See Document II-B of dossier.
(ii) via skin contact	See Document II-B of dossier.
(iii) via drinking water	See Document II-B of dossier.
(iv) via food	See Document II-B of dossier.
(v) indirect via environment	See Document II-B of dossier.

2.10.2 Environmental exposure towards active substance

2.10.2.1 Production

(i) Releases into water	**Production of active substance - Part I:** Technical tebuconazole (Preventol A 8) is produced in [redacted] ([redacted]). Therefore a description of the exposure situation during the production process is not necessary. **Production of active substance - Part II ([redacted]):** Releases into water only occur after cleaning operations. These waters are treated as follows: All water from cleaning processes will be collected. High contaminated cleaning waters will be burned as well as solid waste from the cleaning processes. The resulting water is analysed and - if it meets internal specifications - will go to the industrial STP at [redacted].

Section A2.10 Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Annex Point IIA2.10

(ii) Releases into air	**Production of active substance - Part I:** Technical tebuconazole (Preventol A 8) is produced in [redacted] ([redacted]). Therefore a description of the exposure situation during the production process is not necessary. **Production of active substance - Part II ([redacted]):** Outlet air from the plant is filtered. The isolated dust will be burned
(iii) Waste disposal	**Production of active substance - Part I:** Technical tebuconazole (Preventol A 8) is produced in [redacted] ([redacted]). Therefore a description of the exposure situation during the production process is not necessary. **Production of active substance - Part II ([redacted]):** All solid waste (e.g. filters, contaminated package) will be burned.

2.10.2.2 Intended use(s)

See Document II-B of dossier.

Affected compartment(s):	See Document II-B of dossier.
water	See Document II-B of dossier.
sediment	See Document II-B of dossier.
air	See Document II-B of dossier.
soil	See Document II-B of dossier.
Predicted concentration in the affected compartment(s)	See Document II-B of dossier.
water	See Document II-B of dossier.
sediment	See Document II-B of dossier.
air	See Document II-B of dossier.
soil	See Document II-B of dossier.

Section A2.10 Annex Point IIA2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	8. August 2005
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Competent Authority Report

According to Directive 98/8/EC on Biocidal Products

Tebuconazole

CAS No.: 107534-96-3

ELINCS No.: 403-640-2

Document III-A.3

Physical chemical data of active substance

from Lanxess Deutschland GmbH

for use in wood preservatives (Product type 8)

Reporting Member State: Denmark

May 2007

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point	Directive 92/69/EC,A.1 *RMS: Identical to EEC A1* *DSC*	[redacted]	**result:** 105 °C **pressure:**		[redacted]	[redacted]	Krohn, 1993a	√
3.1.2 Boiling point	*RMS: OECD guideline 113* *TGA*	*RMS:* [redacted]	**result:** **pressure:**	Not measurable, decomposition above 165 °C *RMS:* [redacted]	[redacted]	[redacted]	Mix and Berg, 1988	√
3.1.3 Bulk density/ relative density								
Relative density	OECD guideline 109 Air comparison pycnometer	[redacted]	**result:** 1.25 g/cm^3 at 26 °C	*RMS:* [redacted]	[redacted]	[redacted]	Weber, 1987	√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Bulk density			**result:** approx. 400 kg/m^3	*RMS:* [redacted]	[redacted]	[redacted]	Bayer Chemicals, 2003 (SDS)	-
3.2 Vapour pressure (IIA3.2)	OECD guideline 104	[redacted]	Gas saturation 1.7 · 10^{-6} Pa at 20 °C (extrapolated); 3.9 · 10^{-6} Pa at 25 °C (extrapolated)	*RMS:* [redacted]	[redacted]	[redacted]	Krohn, 1993b	√
	OECD guideline 104	[redacted]	Vapour pressure balance 1.3 · 10^{-6} Pa at 20 °C (extrapolated); 3.1 · 10^{-6} Pa at 25 °C (extrapolated)		[redacted]	[redacted]	Weber, 1988	Not accepted

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.2.1 Henry's Law Constant (Pt. I-A3.2)	Calculated		**Calculated result at 20 °C:** based on Water solubility: 32 mg/L Vapour pressure: $1.3 \cdot 10^{-6}$ Pa $1 \cdot 10^{-5}$ Pa· m^3· mol^{-1}	*RMS:* [redacted]	[redacted]	[redacted]	Krohn, 1988a	√
3.3 Appearance (IIA3.3)								
3.3.1 Physical state	Pure tebuconazole Tech. tebuconazole	[redacted]	Crystals powder		[redacted]	[redacted]	Schneider, 2005a	√
3.3.2 Colour	Pure tebuconazole Tech. tebuconazole	[redacted]	Colourless White to beige		[redacted]	[redacted]	Schneider, 2005a	√
3.3.3 Odour	Pure tebuconazole Tech. tebuconazole	[redacted]	No characteristic odour Slight characteristic odour		[redacted]	[redacted]	Schneider, 2005b	√
3.4 Absorption spectra (IIA3.4)			Structure confirmed by:		[redacted]	[redacted]	Krohn 1988b	√
UV/VIS		*RMS:* [redacted]	UV (methanol), peak maxima= 221.4 nm, Molar absorptivity					√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			[1000 cm^2/mol] = 38.92					
IR		*RMS:* [redacted]	IR (KBr)					√
NMR		*RMS:* [redacted]	^{1}H-NMR (250 MHz, $CDCl_3$) ^{13}C-NMR (63 MHz, $CDCl_3$)					√
MS		*RMS:* [redacted]	MS (electron impact)					√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.5 Solubility in water (IIA3.5)	OECD guideline 105 Flask method	[redacted]	**result:** demin. water: 10 °C: 0.028 g/l, 20 °C: 0.032 g/l, 30 °C: 0.043 g/l; pH 5: 10 °C: 0.022 g/l, 20 °C: 0.027 g/l, 30 °C: 0.035 g/l; pH 7: 10 °C: 0.024 g/l, 20 °C: 0.029 g/l, 30 °C: 0.037 g/l; pH 9: 10 °C: 0.027 g/l, 20 °C: 0.032 g/l, 30 °C: 0.043 g/l; **temperature:** 10 °C / 20 °C / 30 °C **pH:** 5 / 7 / 9	*RMS:* [redacted]	[redacted]	[redacted]	Erstling, 2002	√
3.6 Dissociation constant (-)	OECD guideline 112	[redacted]	It is not possible to specify a pKa value in water	Tebuconazole is a very weak base which can only be completely protonized in non aqueous systems in the presence of very	[redacted]	[redacted]	Placke, 1987	√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
				strong acids.				
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	CIPAC MT 157 CIPAC MT 181 Flask method	[redacted]	**temperature:** 10 °C 2-Propanol: 89.3 g/l, Toluene: 46.9 g/l, n-Hexane: 0.543 g/l, Acetone: 222 g/l, Acetonitrile: 61.9 g/l, 1,2-dichloroethane: 205 g/l, Octanol: 95.5 g/l, **temperature:** 20 °C n-Hexane: 0.841 g/l, Octanol: 98.1 g/l, **temperature:** 30 °C 2-Propanol: 140 g/l, Toluene: 107 g/l, n-Hexane: 1.36 g/l, Acetone: 403 g/l, Acetonitrile: 172 g/l, 1,2-dichloroethane: 322 g/l,		[redacted]	[redacted]	Jungheim, 2005a Jungheim, 2005b	√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			Octanol: 126 g/l,					
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)				Not applicable (the active substance as manufactured didn't include any organic solvent)				√
3.9 Partition coefficient n-octanol/water (IIA3.6)	CIPAC MT 157 CIPAC MT 181 Flask method	[redacted]	**temperature:** 10 °C Pow = 3411, log Pow = 3.53 **temperature:** 20 °C Pow = 3066 log Pow = 3.49 **temperature:** 30 °C Pow = 2930 log Pow = 3.47 **pH:** not investigated because there is no influence of pH on the water solubility		[redacted]	[redacted]	Jungheim, 2005b	√
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	OECD guideline 113	[redacted]	DTA measurement: Exothermal above 350 °C. TGA measurement: A weight loss was observed above 165 °C.		[redacted]	[redacted]	Mix and Berg, 1988	√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			Tebuconazole may be considered stable at room temperature.					

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)								√
Flammability	Directive 92/69/EC, A.10	[redacted]	The test substance is not highly flammable		[redacted]	[redacted]	Müller, 1991	√
Pyrophoric properties	Directive 92/69/EC, A.13	[redacted]	It has no pyrophoric property		[redacted]	[redacted]	Müller, 1991	√
Auto-Flammability	Directive 92/69/EC, A.16	[redacted]	No auto-flammability up to the melting point at 105°C.		[redacted]	[redacted]	Müller, 1991	√
Flammability, water contact			From the structural formula and composition of the substance it can be concluded that the substance does not evolve any flammable gases in contact with water or humid air.					
3.12 Flash-point (IIA3.9)				Not applicable melting point above 40 °C				√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.13 Surface tension (IIA3.10)	OECD guideline 115	[redacted]	**result:** 64.26 mN/m **temperature:** 20 °C concentration 28.8 mg/L	Tebuconazole is classified to be non-surface active	[redacted]	[redacted]	Imre, 1989	√
3.14 Viscosity (-)			**result:** **temperature:**	n.a., active substance is a solid				√
3.15 Explosive properties (IIA3.11)	Directive 92/69/EC, A.14	[redacted]	The test substance is not explosive		[redacted]	[redacted]	Eberz, 1999	√
3.16 Oxidizing properties (IIA3.12)			The structural formula of tebuconazole contains none of the functional groups quoted in "Recommendations On The Transport Of Dangerous Goods- manual of test and Criteria, fourth revised edition, Appendix 6, Chapter 6" which may indicate oxidising properties. Therefore, it can be concluded that the test substance has no oxidising properties.		[redacted]	[redacted]	Heinz, 2005	√

Section A3 Physical and Chemical Properties of Active Substance

Annex Point IIA3.1-3.13

	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.17 Reactivity towards container material (IIA3.13)	EPA pesticide guideline, subdivision D, 63-13	[redacted]	Tebuconazole is compatible with glass, C-4 clear phenolic coated steel, brass, stainless steel, aluminium, kraft paper and polyethylene. It is not compatible with plain tinplate and plain steel.		[redacted]	[redacted]	Talbott, 1988	√

Section A3.8 Annex Point IIIA.3.2.	Stability in organic solvents used in biocidal products and identity of relevant breakdown products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Active substance: Data on the stability of the active in organic solvents are available from the stability test on the guide recipe JJT 3582 which is a solvent based product which contains about 90 % white spirit, mixture of n-, iso-, and cyclic aliphatic compounds (C_{10}-C_{12}). The active was stable for 8 weeks at 40°C (see document II-B, table 1.4, section B3.7). In addition due to the chemical structure of tebuconazole instability of the active in common organic solvents is not to be expected.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A3.14 Annex Point (-)	Viscosity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Because tebuconazole is a solid, determination of viscosity does not apply.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Competent Authority Report
According to Directive 98/8/EC on Biocidal Products

Tebuconazole

CAS No.: 107534-96-3
ELINCS No.: 403-640-2

Document III-A.4

Analysis methods of active substance

from Lanxess Deutschland GmbH
for use in wood preservatives (Product type 8)

Reporting Member State: Denmark

May 2007

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of pure active substance

			Official use only
		1 REFERENCE	
1.1	**Reference**	G. Kulinna, 1994, Folicur, Industrial Active Component, Assay – Capillary Gas Chromatography, Bayer AG, ZF-DZA/Analytik Dormagen, Germany, Analytical method No.: 2201-0274001-94E (unpublished), 1994-04-15 D.I.E. Nonn, 2001, Validation of GLC-method 2201-0274001-94 – Determination of Tebuconazole (Folicur), Industrial, Bayer AG, ZF-Zentrale Analytik Dormagen, Germany, Report No. VB1-2201-0274001 (unpublished), 2001-08-27	√
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	√
2.1	**Guideline study**	Validation was performed in accordance with Directive 91/414/EEC, Annex II and III; Directive 96/46/EC Analytical methods.	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	√
3.1	**Preliminary treatment**		
3.1.1	Enrichment	[redacted]	
3.1.2	Cleanup	[redacted]	
3.2	**Detection**		
3.2.1	Separation method	[redacted]	

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of pure active substance

[redacted]

3.2.2 Detector

3.2.3 Standard(s)

3.2.4 Interfering substance(s)

3.3 Linearity

3.3.1 Calibration range

3.3.2 Number of measurements

3.3.3 Linearity

3.4 Specificity: interfering substances

3.5 Recovery rates at different levels

3.5.1 Relative standard deviation

3.6 Limit of determination

3.7 Precision

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of pure active substance

3.7.1	Repeatability	[redacted]
3.7.2	Independent laboratory validation	No data

√

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

A method to determine the assay of Folicur (tebuconazole) in industrial active component was developed.

The method is based on capillary gas chromatography using flame ionisation detector. The quantitative evaluation is carried out according to the method of the internal standard.

4.2 Conclusion

The method was employed with success on tebuconazole industrial and was found to be valid.

4.2.1 Reliability [redacted]

4.2.2 Deficiencies [redacted]

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of pure active substance

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of tebuconazole residues in surface water

Official use only

5 REFERENCE

5.1 Reference

R.D. Weeren and S. Pelz, 2000, Validation of an analytical method (analogous to DFG method W 5) for the determination of residues of tebuconazole in surface water. Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Specht & Partner No.: BAY-9921V Az. T3303/99. Bayer Method No. 00054 / M003, February 03, 2000

DFG Method W 5: R. Brennecke, K. Vogeler in: Manual of Pesticides Residue Analysis, edited by Thier HP and Zeuner H, Weinheim, New York, 1992, Vol.2, p. 377-386

5.2 Data protection [redacted]

5.2.1 Data owner [redacted]

5.2.2 Companies with letter of access [redacted]

5.2.3 Criteria for data protection [redacted]

6 GUIDELINES AND QUALITY ASSURANCE

6.1 Guideline study

Guidance document 8064/VI/97 rev. 4 of 15.12.98 of the European Commission. BBA Guidelines: Residue analytical methods for post registration control purposes of July 21, 1998

6.2 GLP [redacted]

6.3 Deviations [redacted]

7 MATERIALS AND METHODS

7.1 Preliminary treatment

7.1.1 Enrichment
3.1.2 Cleanup

Method P-14.118 (analogous to DFG Method W 5):
The water sample is extracted three times with dichloromethane. The organic phases are filtered through sodium sulfate. The combined filtrates are evaporated. The residue is dissolved in ethyl acetate and cyclohexane. An aliquot of this solution is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1+1) as eluant and an automated gel permeation chromatograph. The concentrated solution is analysed for tebuconazole by gas chromatography in accordance with the indicated conditions.

Evaluation:
Concentrations of tebuconazole in sample extracts were determined by comparing the detector response (peak height in counts) of the sample with the pertinent detector response obtained from the neighbouring external standard.

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of tebuconazole residues in surface water

7.2	**Detection**	
7.2.1	Separation method	The concentrated solution is analysed for tebuconazole by gas chromatography under the following conditions: Column: fused silica capillary column (DB-5 MS), length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 µm; Gases: Carrier: helium, 1 ml/min; Temperatures: Oven: initial 60 °C (hold for 1 min), heat rate 40 °C/min to 100 °C, heat rate 10 °C/min to 250 °C (hold for 10 min), Injector: 250 °C, Interface: 280 °C; Injection volume: 1 µl
7.2.2	Detector	Mass selective detector (MSD), Selected ions: m/z 250 (quantitation), m/z 125, 252, 127 (verification)
7.2.3	Standard(s)	External standard (tebuconazole)
7.2.4	Interfering substance(s)	Water from the German river Alster (pH 8.2, DOC: 5.2 mg/l, total hardness 11°dH, TOC: 20 mg/l, mud content: 50 mg/l)
7.3	**Linearity**	
7.3.1	Calibration range	Single measurement of 8 concentrations (see table 4_2-1)
7.3.2	Number of measurements	Single measurement of 8 concentrations (see table 4_2-1)
7.3.3	Linearity	Correlation coefficient r = 0.9994 (r^2 = 0.9988)
7.4	**Specificity: interfering substances**	No significant interferences from the sample matrix were detected at the retention time corresponding to tebuconazole in any of the control samples.
7.5	**Recovery rates at different levels**	Fortification levels of the surface water were 0.05 µg/l and 0.5 µg/l. Control samples were analysed in duplicate. Fortified samples were analysed in quintuplet for each fortification level. Mean recoveries were 97% for the 0.05 µg/l fortification level with single values ranging from 85 to 105% and 86% for the 0.5 µg/l fortification level with single values ranging from 75 to 93%.
7.5.1	Relative standard deviation	8.0% for 0.05 µg/l and 7.8% for 0.5 µg/l
7.6	**Limit of determination**	Limit of quantitation (LOQ): 0.05 µg/l Limit of detection (LOD): 0.02 µg/l
7.7	**Precision**	
7.7.1	Repeatability	See recovery rates (3.5). Each five samples were analysed.
7.7.2	Independent laboratory validation	The validation was performed by an external laboratory (see line 1.1: "Reference")

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of tebuconazole residues in surface water

8 APPLICANT'S SUMMARY AND CONCLUSION

8.1 Materials and methods

A validation of the analytical method P-14.118 (analogous to DFG Method W 5) was performed in surface water for environmental and legally relevant concentrations between 0.05 and 0.5 µg/l. The method is based on gas chromatography analysis on fused silica gel with a mass specific detector. Prior to the gas chromatographic analysis an extraction with dichloromethane is performed.

8.2 Conclusion

The accuracy was considered acceptable since the results were in the range 70-110%. All the results obtained using this method were within this range. The precision results should be better than 20% over the range covered. The precision data obtained fall within these limits. The analytical method P-14.118 (analogous to DFG Method W 5) permits the reliable determination of residues of tebuconazole in surface water over the range 0.05 to 0.5 µg/l.

8.2.1 Reliability

Reliability indicator = [redacted]

8.2.2 Deficiencies

[redacted]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date July 2005

Materials and methods [redacted]

Conclusion [redacted]

Reliability [redacted]

Acceptability [redacted]

Remarks [redacted]

COMMENTS FROM ...

Date Give date of comments submitted

Results and discussion Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.
Discuss if deviating from view of rapporteur member state

Conclusion Discuss if deviating from view of rapporteur member state

Reliability Discuss if deviating from view of rapporteur member state

Acceptability Discuss if deviating from view of rapporteur member state

Remarks

Table 4_2-1: Linearity of mass selective detector response of tebuconazole

External standard concentration (mg/l)	Peak height (count)
0.00503	172
0.0134	470
0.0335	1100
0.0670	2500
0.134	5100
0.251	11000
0.503	23600
0.670	31000

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of tebuconazole residues in air

Official use only

1 REFERENCE

1.1 Reference

K. Riegner, 1992, Method for the determination of Tebuconazole in air, Bayer AG, Institute for Product information and residue analysis, Leverkusen, Germany, Report No. RA-605/92, Method No. 00278 (unpublished), 1992-10-27

E. Hellpointner, 2000, Confirmatory Method for the Determination of Tebuconazole in air (confirmed method: 00278), Bayer AG, Institute for Metabolism Research and Residue Analysis, Leverkusen, Germany, Report No. MR470/00 (unpublished), 2000-10-19

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No,

no guideline available

2.2 GLP

The confirmatory method for the determination of tebuconazole in air was performed in accordance with the GLP requirements.

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

3.1.2 Cleanup

The adsorption tube contains two adsorption layers being separated by cotton wool with the larger layer facing the inlet of the tube during air sampling. The second, smaller adsorption layer is used to check whether active ingredient has possibly penetrated the larger layer during sampling. For sampling air is sucked through Tenax or XAD-2 adsorption tubes at a flow rate of 2 l/min over a period of 6 hours (V = 720 l). After sampling the tubes are closed with plastic caps and stored under cool conditions.
For extraction of the active ingredient from the adsorption material, both adsorption layers are extracted separately with the cotton wool separating the layers and the upper wool facing the inlet of the tube being analysed together with the first, larger layer. The individual layers are removed from the glass tube and put into a 5 ml sprung lid bottle. The glass tube is rinsed with 3 ml ethyl acetate. These 3 ml solvent are added to the first (large) layer and 2 ml ethyl acetate are added to the second (small) layer. The active ingredient is extracted from the adsorption material by ultrasonication for 10 minutes. An amount of the clear solution is analysed by gas chromatography according to the indicated conditions.

Quantitative evaluation is made by means of an integrator by determination and comparison of the peak areas of standard solutions with the peak areas of the analytical solutions (external standard method). Each solution is analysed twice and the respective mean value is used for the calculation.

For the confirmatory procedure which is based on gas chromatography using mass selective detection no deviation from the described Tenax sampling and extraction technique is necessary. The same crude extracts could be investigated by both different GC methods.

3.2 Detection

3.2.1 Separation method

The determination is performed by capillary gas chromatography under the following chromatographic conditions:

GC-NPD conditions:
Column: Chrompack; CP-WAX 52 CB (length: 10 m, inner diameter: 0.25 mm, film thickness: 0.20 µm);
Injector:
Cold application system,
Temperature program:
Step 0: 10 sec
Step 1: 50 °C
Step 2: 5 °C/sec
Step 3: 85 °C
Step 4: 30 sec
Step 5: 12 °C/sec
Step 6: 270 °C
Step 7: 120 sec
Step 8: 100 °C, operation mode with solvent stop out
Step 9: 6 min;

Splitless time: 0.65 min up to 2.00 min; Injection volume: 1 µl;
Carrier gas: helium, delivery pressure = 0.9 bar;
Total flow rate: 45 ml/min (RT);
Oven temperature: T1 = 80 °C, t1 = 1.0 min,
rate-1 = 25 °C/min;
T2 = 250 °C, t2 = 2.2 min

GC-MSD conditions:
Column: HP-1701 (15% CNP-PH Me Siloxane), length: 25 m, inner diameter: 0.2 mm, film thickness: 0.20 µm;
Injector: cold injection system;

Injector conditions:
Split mode: splitless, Initial temp.:55 °C, Initial time: 0.10 min;
1st rate: 12.0 °C/sec, 1st final temp.: 325 °C,
1st final time: 5.00 min, Cryo cooling: OFF,
Splitless time: 1.10 min, Purge time: 0.70 min,
Equilib time: 0.00 min;

Carrier gas: helium, Constant flow pressure: 65 kPa, Constant flow: 0.6 ml/min;

Column gradient:

Section A4 (4.1-4.3) **Analytical Methods for Detection and Identification**

Annex Point IIA4.1/4.2 & IIIA-IV.1 Analytical method for the determination of tebuconazole residues in air

		Level / Rate [°C/min] / Temp. [°C] / Final time [min] Initial / – / 150 / 0 1 / 50 / 280 / 5 Injection volume: 2 µl
3.2.2	Detector	Nitrogen and phosphorous detector (NPD, 300 °C). For confirmation mass selective detection (MSD) was used (SIM mode, three individual ions (m/z 250, 125 and 70) were used for detection)
3.2.3	Standard(s)	External standard (tebuconazole)
3.2.4	Interfering substance(s)	Substances of the adsorption material
3.3	**Linearity**	
3.3.1	Calibration range	The detector linearity was checked in the range from 0.017 to 1.701 mg/l. Confirmatory method: the detector linearity was checked in the range from 0.0872 to 0.8715 mg/l.
3.3.2	Number of measurements	Single measurement of nine concentrations; Confirmatory method: single measurement of five concentrations
3.3.3	Linearity	Coefficient of determination: 0.998640; Confirmatory method: Correlation coefficient: 0.9920.
3.4	**Specificity: interfering substances**	No significant interferences found. Confirmatory method: blank values of control samples were about 1% of the LOQ level.
3.5	**Recovery rates at different levels**	The recovery rates were checked by spiking adsorption tubes with active ingredient (dissolved in ethyl acetate). The solvent was removed by sucking air through the tube (2 l/min) for about 10 minutes. Subsequently the adsorption tubes were exposed to defined climatic conditions (e.g. in a climatic cabinet). After a short equilibration phase (approx. 20 min) air being conditioned correspondingly was sucked through the adsorption tubes at a rate of 2 l/min over a period of 6 hours. Fortification experiments were performed over the range from 0.0011 to 0.142 mg a.i. /m³. Results were obtained from four tests for each fortification level. The mean recovery rates were in the range of 94 to 104% with relative standard deviations of 2.0 to 6.3% depending on the amount of active ingredient and the climatic conditions. Range of data, fortification levels and climatic conditions see table A4_2-1a and table A4_2-1b. Confirmatory method: two fortification experiments were performed at 0.0011 mg a.i. /m³. The concentration of tebuconazole in the two spiked samples (evaluated against the linearity) could be calculated with 0.3175 and 0.3235 µg/ml, representing a mean recovery of 110% of the spiked amount in theory.
3.5.1	Relative standard deviation	Relative standard deviations: 2.0% to 6.3% depending on the type of adsorption material, the amount of active ingredient and the climatic conditions.

Section A4 (4.1-4.3) **Analytical Methods for Detection and Identification**

Annex Point IIA4.1/4.2 & IIIA-IV.1 Analytical method for the determination of tebuconazole residues in air

Detailed results see table A4_2-1a and table A4_2-1b.

3.6 Limit of determination The lower limit of determination was 0.0011 mg a.i./m^3 air.

3.7 Precision

3.7.1 Repeatability: See recovery rates (point 3.5)

3.7.2 Independent laboratory validation: No data

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

A method for the gas chromatographic determination of tebuconazole in air is performed.
Air is sucked through Tenax or XAD-2 adsorption tubes at a rate of 2 l/min during a period of 6 hours. The adsorbed active ingredient is extracted with ethyl acetate and determined after gas chromatographic separation by means of a nitrogen and phosphorous selective detector (GC-NPD).
For this method a confirmatory procedure was developed. It is based on gas chromatography using mass selective detection (GC-MSD). No deviation from the described Tenax sampling and extraction technique is necessary. The same crude extracts could be investigated by both different GC methods.

4.2 Conclusion

The method allows the determination of tebuconazole in air in a concentration range of 0.0011 mg a.i./m^3 (= lower limit of determination) to 0.142 mg a.i./m^3, with two different adsorption systems being available to choose from. The systems were validated under different climatic conditions. It was shown that the active ingredient could not be desorbed with air from Tenax and XAD-2, neither at low nor at high concentrations, temperatures and humidities.

For confirmation no deviation from the described Tenax sampling and extraction technique is necessary. The same crude extracts could be investigated by both different GC methods, either by detection using NPD or a MSD. The entirely different properties and selectivity of the MS detection justify its use as confirmation method.

4.2.1 Reliability [redacted]

4.2.2 Deficiencies [redacted]

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical method for the determination of tebuconazole residues in air

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and methods	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A4_2-1a Recovery rates (1st layer) in case of adsorption on Tenax

Concentration [mg a.i./m³]	Climatic conditions		Recovery rate [%]	Relative standard deviation [%]
	°C	RH [%]		
0.0011	20	30	97.8 (92.8 – 101)	3.7
0.0011	35	80	104 (99.4 – 106)	2.8
0.142 (*)	35	30	97.1 (89.9 – 104)	6.3

The results were obtained from 4 tests each for the determination of the recovery rate

RH = relative air humidity

Chromatographic blank values in the range from 0.7% to 8.5% may occur relative to the smallest added amount of 0.0011 mg a.i./m³.

(*) The second adsorption layer contained less than 3.5% active ingredient (relative to the added amount of active ingredient)

Table A4_2-1b Recovery rates (1st layer) in case of adsorption on XAD-2

Concentration [mg a.i./m³]	Climatic conditions		Recovery rate [%]	Relative standard deviation [%]
	°C	RH [%]		
0.0011	20	30	94.7 (91.1 – 98.5)	3.4
0.0011	35	80	97.9 (95.3 – 100)	2.1
0.142 (*)	35	30	93.6 (91.8 – 95.2)	2.0

The results were obtained from 4 tests each for the determination of the recovery rate

RH = relative air humidity

Chromatographic blank values in the range from 0.8% to 5.9% may occur relative to the smallest added amount of 0.0011 mg a.i./m³.

(*) The second adsorption layer contained less than 3.5% active ingredient (relative to the added amount of active ingredient)

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1 Analytical method for the determination of tebuconazole residues in soil

Official use only

1 REFERENCE

1.1	**Reference**	Weeren, R.D.and Pelz, S., 2000, Validation of DFG method S 19 (extended revision) for the determination of residues of tebuconazole in soil, Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany, Specht & Partner No.: BAY-0004V, Az. G00-0032, BAYER method No. 00086/E054, 2000-08-22. DFG Method S 19: Specht, W.; in: Organochlorine, organophosphorus, nitrogen-containing and other pesticides, edited by Thier, H. P., Verlag Chemie, Weinheim, 1991
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Guidance document SANCO/825/00 rev. 6 of 20/06/00 of the European Commission; BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	DFG Method S 19
3.1.2	Cleanup	Specimen material is extracted with acetone. Water is added beforehand in an amount that takes full account of the natural water content of the specimen so that during extraction the acetone:water ratio remains constant at 2:1 (v:v). For liquid-liquid partition ethyl acetate + cyclohexane (1+1) and sodium chloride were added. After separation and concentration by evaporation an aliquot of the organic phase is cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate+cyclohexane (1+1) as eluent. The residue containing fraction is concentrated and analysed for residues of tebuconazole by gas chromatography according to the indicated conditions. Evaluation: Concentrations of tebuconazole in sample extracts were determined by comparing the detector response of the injected sample with the pertinent detector response obtained from the neighbouring external standard.
3.2	**Detection**	
3.2.1	Separation method	The concentrated solution is analysed for tebuconazole by gas chromatography using nitrogen/phosphorus detector under the following

		conditions: Column: 30 m fused silica column (DB-5), internal diameter: 0.53 mm, film thickness: 1.5 µm; Gas flow rates: Carrier: helium, 5.0 ml/min, Make up: helium, 30 ml/min, Detector: synth. air: 60 ml/min, hydrogen: 3 ml/min; Temperatures: Oven: initial 150 °C, hold for 1 min, heat rate 10 °C/min to 250 °C, hold for 10 min, heat rate 25 °C/min t 280 °C, hold for 3 min; Injector: 250 °C Injection volume: 5 µl, splitless; Conditions for mass selective detector: Column: 30 m fused silica column (DB-5), internal diameter: 0.25 mm, film thickness: 0.25 µm; Gas: Carrier: helium, 2.3 ml/min; Temperatures: Oven: initial 60 °C, hold for 1 min, with a rate of 20 °C/min to 250 °C, hold for 15 min; Injector: 250 °C Interface: 280 °C; Injection volume: 1 µl, splitless;
3.2.2	Detector	Nitrogen/phosphorus detector (NPD, 270 °C) and mass selective detector (MSD, m/z 250 (quantification); m/z 125, m/z 127 and m/z 252 (verification))
3.2.3	Standard(s)	External standard (tebuconazole)
3.2.4	Interfering substance(s)	Soil from Germany (standard soil 2.2, LUFA Speyer) analyzed according to this method and checked for interfering substances
3.3	**Linearity**	
3.3.1	Calibration range	Single measurement of nine concentrations ranging from 0.100 to 2.01 µg/ml (see table 4_2-1)
3.3.2	Number of measurements	Single measurement of nine concentrations (see table 4_2-1)
3.3.3	Linearity	Correlation coefficient r= 1.0000
3.4	**Specificity: interfering substances**	No significant interferences from the sample matrix were detected at the retention time corresponding to tebuconazole in any of the control samples.
3.5	**Recovery at different levels**	Fortification levels of the soil were 0.01 mg/kg and 0.10 mg/kg. Control samples were analysed in duplicate and fortified specimens were analysed in quintuplet. Mean recoveries were 100% for 0.01 mg/kg with single values ranging from 94 – 105%, and 94% for 0.10 mg/kg with single values ranging from 92 – 95%. For confirmation analysis one fortified specimen of each level was analysed. The recoveries obtained were 98% for 0.01 mg/kg and 101% for 0.10 mg/kg.

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1 Analytical method for the determination of tebuconazole residues in soil

3.5.1	Relative standard deviation	4.3% for 0.01 mg/kg and 1.2% for 0.1 mg/kg
3.6	**Limit of determination**	Limit of quantitation (LOQ): 0.01 mg/kg, Limit of detection (LOD): 0.002 mg/kg
3.7	**Precision**	Covered by point 3.5 (recovery).
3.7.1	Repeatability	See recovery rates (point 3.5): Each five samples were analyzed.
3.7.2	Independent laboratory validation	The validation was performed by an external laboratory (see line 1.1: "Reference")
		4 APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Material and methods**	A validation of the analytical method DFG Method S 19 (including a clean-up by gel permeation) was performed with soil samples at environmentally and legally relevant concentrations between 0.01 and 0.1 mg/kg. The method is based on gas chromatography analysis on fused silica gel with a nitrogen/phosphorus detector or mass specific detector.
4.2	**Conclusion**	The accuracy is considered acceptable as the mean recovery is in the range of 70 – 110%. All the results obtained using this method were within this range. The precision results should be better than 20% over the range covered. The precision data obtained fulfilled this limit. The DFG method S 19 (extended version) permits the reliable determination of residues of tebuconazole in soil with satisfactory accuracy and precision over a range from 0.01 to 0.1 mg/kg.
4.2.1	Reliability	Reliability indicator = [redacted]
4.2.2	Deficiencies	[redacted]

Section A4 (4.1-4.3) Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1 Analytical method for the determination of tebuconazole residues in soil

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4_2-1: Linearity of nitrogen/phosphorus detector response of tebuconazole

External standard concentration (µg/ml)	Peak height y (count)
0.0100	0.32
0.0201	0.58
0.0401	1.19
0.0802	2.27
0.160	4.58
0.334	9.74
0.668	19.51
1.34	40.23
2.01	59.65

Section 4.2 (d) Annex Point IIA 4.2(d)	Analytical method for animal and human body fluids and tissues	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	According to the TNGD for data requirements an analytical method for animal and human body fluids and tissues is required for toxic and very toxic actives. Tebuconazole is not classified as toxic or very toxic. Therefore an analytical method for animal and human body fluids and tissues is not a data requirement for tebuconazole in context of the EU BPD dossier for the active.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 4.3 Annex Point IIIA 4.1	Analytical Methods for Residues in Food and Feedstuffs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	[redacted] Due to the non-intended exposure to food and feedstuffs it is justified not to submit an analytical method for residues in food and feedstuffs in the scope of the BPD submission as an active in wood preservatives.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*8. August 2005*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Competent Authority Report
According to Directive 98/8/EC on Biocidal Products

Tebuconazole

CAS No.: 107534-96-3
ELINCS No.: 403-640-2

Document III-A.5

Efficacy of active substance

from Lanxess Deutschland GmbH
for use in wood preservatives (Product type 8)

Reporting Member State: Denmark

May 2007

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Fungicide used both in agriculture and wood preservation	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	See attached **Summary Table A5.1**, See also Study Summaries for section 5.3.1 (Effects on target organisms).	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	In particular wood rotting fungi (basidiomycota)	
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	Protection of wooden articles and structures	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**		
5.3.1	**Effects on target organisms (IIA5.3)**	See attached **Summary Table A5.1**, See also Study Summaries for section 5.3.1 (Effects on target organisms).	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**		
	PT8	Wood preservative: Up to 0.6 % tebuconazole in formulated wood preservatives (water based and solvent-based formulations)	
	PTn		
5.4	**Mode of action (including time delay) (IIA5.4)**		
5.4.1	**Mode of action**	As a fungicide, tebuconazole interferes with basic metabolism of the fungal cell wall and contents.	
5.4.2	**Time delay**	Not relevant this kind of application (wood preservation)	
5.5	**Field of use envisaged (IIA5.5)**		

Section A5 Effectiveness against target organisms and intended uses

	MG01: Disinfectants, general biocidal products	
	MG02: Preservatives MG03: Pest control MG04: Other biocidal products Further specification	Product type PT08: Wood preservatives
5.6	**User (IIA5.6)**	
	Industrial i) Open system ii) Closed system	See Documents II-B and II-C of dossier.
	Professional i) Open system ii) Closed system	See Documents II-B and II-C of dossier.
	General public	See Documents II-B and II-C of dossier.
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	
5.7.1	**Development of resistance**	For industrial wood preservation using tebuconazole this is not an issue. Resistance is usually associated with continued application and resistance is formed between applications such that subsequent applications are less efficacious. Industrial wood preservatives are usually applied only once and there is no evidence to suggest resistance. Also, for other kinds of wood preservation with tebuconazole-containing products, cases of resistances are not reported or known up to the time being.
5.7.2	**Management strategies**	Not relevant
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	See entries in IUCLID database

Table A5.1: Summary Table: Experimental data on the effectiveness of the active substance against target organisms

Test substance	Test organism(s)	Test system / concentrations applied / exposure time	Test results: effects, mode of action, resistance	Reference*
Preventol A 8 (purity [redacted] tebuconazole)	**Ascomycota/Deuteromycota:** *Penicillium brevicaule, Chaetomium globosum, Aspergillus niger, Aureobasidium pollulans, Alternaria tenuis, Cladosporium herbarum, Dothichiza pityophila, Rhodotorula rubra, Fusarium solani, Geotrichum candidum, Paecilomyces variotii Brainer, Ceratocystis pilifera, Phialophora fastigiata, Aspergillus ustus, Stachybotris chartarum, Penicillium citrinum, Aspergillus flavus Link* **Basidiomycota:** *Gloeophyllum trabeum, Coniophora puteana, Poria placenta, Lentinus tigrinus, Coriolus versicolor, Stereum sanguinolentum*	Determination of minimum inhibiting concentrations (MIC) in agar nutrient medium	**Ascomycota/Deuteromycota:** MIC = 400 ppm: *Paecilomyces variotii Brainer* MIC = 200 ppm: *Rhodotorula rubra* MIC = 100 ppm: *Penicillium brevicaule, Alternaria tenuis, Fusarium solani, Geotrichum candidum, Stachybotris chartarum, Penicillium citrinum* MIC = 50 ppm: *Aspergillus flavus Link* MIC = 35: *Phialophora fastigiata* MIC = 20 ppm: *Cladosporium herbarum, Ceratocystis pilifera* MIC = 10 ppm: *Chaetomium globosum, Aspergillus niger, Aureobasidium pollulans, Aspergillus ustus* MIC < 1 ppm: *Dothichiza pityophila* **Basidiomycota:** MIC = 3 ppm: *Coniophora puteana, Stereum sanguinolentum* MIC = 1 ppm: *Poria placenta* MIC = 0.5 ppm: *Gloeophyllum trabeum, Coriolus versicolor,* MIC = 0.3 ppm: *Lentinus tigrinus*	Kugler (2003)

Table A5.1: Summary Table: Experimental data on the effectiveness of the active substance against target organisms (continued)

Test substance	Test organism(s)	Test system / concentrations applied / exposure time	Test results: effects, mode of action, resistance	Reference*
BSA 2069 ([redacted] tebuconazole, [redacted])	Basidiomycetes: *Coniophora puteana, Gloeophyllum trabeum, Poria placenta*	Preventive efficacy against wood destroying Basidiomycetes in pattern from EN 113 (modified according to RAL-GZ 830) after evaporative ageing (EN 73, 1989) -Wood species: *Pinus sylvestris* test blocks -Treatment: preservative brushing procedure - Amount applied: 210-220 ml/m², undiluted product Exposure time: according to EN 113	**Losses in mass (%):** *Coniophora puteana:* 0.0-0.8 (untreated control: 38.8), *Gloeophyllum trabeum:* 0.0-2.1 (untreated control: 35.4), *Poria placenta:* 1.0-1.7 (untreated control: 39.2). **Macroscopic assessment of the specimens after cleavage:** None of the treated specimens were destroyed by the fungi.	Janotta and Melzer (1992)
JJT 3008 ([redacted] Tebuconazole)	*Coniophora puttana, Gloeophyllum trabeum, Poria placenta, Coriolus versicolor*	Preventive efficacy against wood destroying fungi according to EN 113 after ageing by a leaching procedure (EN 84, 1997) -Wood species: *Pinus sylvestri, Fagus sylvatica* (for C. versicolor only) -Treatment: vacuum pressure - Concentrations applied: 0 - 1.1 mass% (*Pinus s.*) 0 - 1.2 mass% (*Fagus s.*) -Exposure: according to EN 113	The test formulation was efficacious at preventing wood destruction by four different fungi on artificially aged pine and beech wood. Mass loss of specimens treated with the lowest dose of tebuconazole was 0.1-1.4%, while the mass loss of untreated controls was 21.1–37.7%. The lowest protective test concentration that was effective was 0.2% w/w equivalent to 1.2 kg TS/m^3 or 120 g a.i./m^3.	Schumacher and Wessely (2002a)
JJT 3008 ([redacted] Tebuconazole)	*Coniophora puttana, Gloeophyllum trabeum, Poria placenta, Coriolus versicolor*	Preventive efficacy against wood destroying fungi according to EN 113 after a ageing by evaporation (EN 73 1990) -Wood species: *Pinus sylvestri, Fagus sylvatica* (for C. versicolor only) -Treatment: vacuum pressure - Concentrations applied: 0 - 1.1 mass% (*Pinus s.*)	The test formulation was efficacious at preventing wood destruction by four different fungi on artificially aged pine and beech wood. The highest non-protective concentrations were 0.3-0.5% on pine and 0.3-0.6% on beech, respectively. Thus, for pine the lowest protective concentration was 0.5% w/w eq. to 3.0 kg TS/m^3 or 300 g a.i./m^3. For beech, the lowest protective	Schumacher and Wessely (2002b)

		0 - 1.2 mass% (*Fagus s.*) -Exposure: according to EN 113	concentration was 0.6% w/w eqv. to 3.4 kg TS/m^3 or 340 g a.i./m^3 softwood.	

*References: See next page

References:

Kugler, M. (2003) Determination of the antimicrobial effects of Preventol A 8 against fungi. Bayer Chemicals AG, Test Report 2003-04-14, 2003-04-16 (unpublished).

Janotta, O. and Melzer, H. (1992): Test of the preventive efficacy of wood preservatives against wood destroying Basidiomycetes in pattern from EN 113 after evaporative ageing. ÖHFI (Austrian Wood Research Institute), Order No. 1058/92/P 1, 1992-10-29 (unpublished).

Schumacher, P., and Wessely, H. (2002) Test report Nr. 3.2/01/8208/01. Materialprüfungsamt des Landes Brandenburg, Report No. 3.2/01/8208/01, 2002-05-23 (unpublished).

Schumacher, P., and Wessely, H. (2002) Test report Nr. 3.2/01/8208/02. Materialprüfungsamt des Landes Brandenburg, Report No. 3.2/01/8208/01, 2002-07-19 (unpublished).

Section A5.3 Efficacy Data
Annex Point IIA5.3 5.3.1. Effects on target organisms

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kugler, M. (2003) Determination of the antimicrobial effects of Preventol A 8 against fungi. Bayer Chemicals AG, Test Report 2003-04-14, 2003-04-16 (unpublished).	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with Letter of Access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
1.3	**Guideline study**	[redacted]	
1.4	**Deviations**	–	
		2 METHOD	
2.1	**Test Substance**	As described in Section 2 of dossier.	
2.1.1	Purity	[redacted] tebuconazole; [redacted]	
2.1.2	Physical state and nature	White to pale brown, crystalline powder.	
2.1.3	Method of analysis	As described in Section 4 of dossier.	
2.2	**Reference substance**	No	
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	See Table 1.1	
2.3.2	Test system	Growth inhibition in agar media.	
2.3.3	Application of TS	Stock solutions of the test substance are added to the growth medium to yield the desired final concentrations.	
2.3.4	Test conditions	Ascomycetes and Deuteromycetes: The solidified nutrient substrate is inoculated with 50 µL spore suspension . Basidiomycetes: Once the agar has solidified, one piece of mycelium is placed on the nutrient substrate containing the test substance, at the middle of the edge of each dish segment, using a sterile inoculation needle. Furthermore, six control samples are also prepared without the addition of test substances.	
2.3.5	Duration of the test / Exposure time	All dishes are incubated at 26 °C ± 2 °C and approx. 70 % humidity. Ascomycetes and Deuteromycetes 14 days Basidiomycetes 4 - 5 days	
2.3.6	Number of replicates performed	–	

Section A5.3 **Efficacy Data**
Annex Point IIA5.3 5.3.1. Effects on target organisms

2.3.7	Controls	A growth control sample is prepared for each germ. Any contamination of the nutrient substrates is detected by simultaneously testing non-inoculated control samples of the nutrient media used.
2.4	**Examination**	
2.4.1	Effect investigated	Fungal growth
2.4.2	Method for recording / scoring of the effect	The growth of the fungi in the test dishes is compared with that of the control. The lowest concentration at which no growth can be detected is stated as the minimum inhibitory concentration (MIC).
2.4.3	Intervals of examination	Fungal growth is evaluated after the incubation time (see 2.3.5) is elapsed.
2.4.4	Statistics	–
2.4.5	Post monitoring of the test organism	No
	3 RESULTS	
3.1	**Efficacy**	
3.1.1	Dose/Efficacy curve	–
3.1.2	Begin and duration of effects	–
3.2	**Tabular and/or graphical presentation of the summarised results**	See Table 1.2
3.3	**Efficacy limiting factors**	
3.3.1	Occurrences of resistances	–
3.3.2	Other limiting factors	–
	4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	The applied laboratory method allows high-throughput screening for potential target organisms.
4.2	**Intended actual scale of biocide application**	–
4.3	**Relevance compared to field conditions**	
4.3.1	Application method	No, not relevant.
4.3.2	Test organism	Yes, the screened organisms have wood-destroying properties.
4.3.3	Observed effect	Yes, microbial growth was investigated under optimum conditions for the respective organisms. Growth conditions on actual wood substrates will be substantially poorer. The estimated MIC values should thus be more than sufficient to prevent fungal growth on wood in service.

Section A5.3 Efficacy Data
Annex Point IIA5.3 5.3.1. Effects on target organisms

4.4	**Relevance for read-across**	Yes
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The inhibitory effect of test substances on material-destroying fungi of practical relevance is determined in the primary screening. The goal of the primary screening is to determine the antifungal inhibitory concentration.
5.2	**Reliability**	2
5.3	**Assessment of efficacy, data analysis and interpretation**	The MICs of tebuconazole against the tested Basidiomycota were in the range between 0.3 ppm and 3 ppm. For the tested Ascomycota and Deuteromycota, MICs in the range between < 1 ppm and 400 ppm were determined.
5.4	**Conclusion**	Tebuconazole has an excellent activity against wood-destroying Basidiomycota and inhibits also all examined Ascomycota and Deuteromycota.
5.5	**Proposed efficacy specification**	–

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	3 August 2005
Comments	[redacted]
Summary and conclusion	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

Table 1.1 Population / Inoculum

Organism	Culture designation
Ascomycota and Deuteromycota	
Penicillium brevicaule	S 3*
Chaetomium globosum	ATCC 62055
Aspergillus niger	ATCC 10575
Aureobasidium pullulans	ATCC 15233
Alternaria tenuis	S 1910*
Cladosporium herbarum	S 1900*
Dothichiza pityophila	S 149
Rhodotorula rubra	H 29*
Fusarium solani	IMI 314228
Geotrichum candidum	IMI 321760
Paecilomyces variotii Brainer	S 800*
Ceratocystis pilifera	S 1750*
Phialophora fastigiata	S 1770*
Aspergillus ustus	S 920*
Stachybotrys chartarum	ATCC 16026
Penicillium citrinum	S 20*
Aspergillus flavus Link	S 700*
Basidiomyceta	
Gloeophyllum trabeum	BAM 109
Coniophora puteana	BAM Ebw 15
Poria placenta	TPRL280
Lentinus tigrinus	ATCC 11779
Coriolus versicolor	CTB 863A
Stereum sanguinolentum	ATCC 32895

* Internal designation of Bayer AG

Tables for Results

Table 1.2 Minimum inhibitory concentrations (MIC)

Organism	MIC [ppm]
Ascomycota and Deuteromycota	
Penicillium brevicaule	100
Chaetomium globosum	10
Aspergillus niger	10
Aureobasidium pullulans	10
Alternaria tenuis	100
Cladosporium herbarum	20
Dothichiza pityophila	< 1
Rhodotorula rubra	200
Fusarium solani	100
Geotrichum candidum	100
Paecilomyces variotii Brainer	400
Ceratocystis pilifera	20
Phialophora fastigiata	35
Aspergillus ustus	10
Stachybotrys chartarum	100
Penicillium citrinum	100
Aspergillus flavus Link	50
Basidiomyceta	
Gloeophyllum trabeum	0.5
Coniophora puteana	3
Poria placenta	1
Lentinus tigrinus	0.3
Coriolus versicolor	0.5
Stereum sanguinolentum	3

Section A5.7.2 Annex Point (-)	Management strategies with respect to development of resistances	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [..]** **Other justification [X]**	
Detailed justification:	Under point 7.1 (development of resistances) is stated in document IIIA5: *For industrial wood preservation using tebuconazole this is not an issue. Resistance is usually associated with continued application and resistance is formed between applications such that subsequent applications are less efficacious. Industrial wood preservatives are usually applied only once and there is no evidence to suggest resistance. Also, for other kinds of wood preservation with tebuconazole-containing products, cases of resistances are not reported or known up to the time being.* [redacted]	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8 August 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.1.1

Annex Point IIA6.1.1

6.1 Acute Toxicity

Acute oral toxicity to the rat (LD_{50})

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], HWG 1608 Technical – Acute oral toxicity study on rats, [redacted], Report No. [redacted], 1991-12-03
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Japanese MAFF Guideline (59, Nohsan no. 3850)
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608 technical
3.1.1	Lot/Batch number	816096181
3.1.2	Specification	technical grade
3.1.2.1	Description	white powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Dosing solutions were freshly prepared on the day of application.
3.2	**Test Animals**	
3.2.1	Species	rat
3.2.2	Strain	Sprague-Dawley (Crj:CD)
3.2.3	Source	[redacted]
3.2.4	Sex	males and females (1:1)
3.2.5	Age/weight at study initiation	age: seven weeks weight: 189-206 g (males) and 161-182 g (females)
3.2.6	Number of animals per group	five per sex per dose
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days

Section A6.1.1

Annex Point IIA6.1.1

6.1 Acute Toxicity

Acute oral toxicity to the rat (LD_{50})

		Oral
3.3.2	Type	gavage
3.3.3	Concentration	1600, 2300, 3000, 3900 and 5000 mg/kg body weight (males) 730, 950, 1230, 1600, 2300, 3000, 3900 and 5000 mg/kg body weight (females)
3.3.4	Vehicle	Suspension in polyethylene glycol 400
3.3.5	Concentration in vehicle	dependent on dose group
3.3.6	Total volume applied	1 ml per 100 g body weight
3.3.7	Controls	-
3.4	**Examinations**	Clinical observations, necropsy
3.5	**Method of determination of LD_{50}**	Bliss
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	[redacted]
4.2	**Pathology**	[redacted]
4.3	**Other**	
4.4	**LD_{50}**	males: 4000 mg/kg bw (3300 – 5800 mg/kg bw) females: 1700 mg/kg bw (1400-2200 mg/kg bw)

Section A6.1.1

Annex Point IIA6.1.1

6.1 Acute Toxicity

Acute oral toxicity to the rat (LD_{50})

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to Japanese MAFF Guideline which is comparable to OECD Guideline 401. The LD50 was established for males and females, respectively.

5.2 Results and discussion

The test substance showed slight to moderate oral toxicity in rats.

The observed clinical symptoms were considered to be similar to those of central nervous system depressant such as anaesthetic agent.

There was no sex difference in observed symptoms, the onset and the disappearance time of the symptoms and death. Abnormal findings in the liver were considered to be due to the test substance because these were observed dose-dependently. Because the findings in the urinary bladder, the adrenals and the trachea were observed only in a few animals, these were not considered to be due to the test substance administration.

5.3 Conclusion

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_1-1. Table for Acute Toxicity

Dose [mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death
males			
1600	[redacted]	[redacted]	[redacted]
2300	[redacted]	[redacted]	[redacted]
3000	[redacted]	[redacted]	[redacted]
3900	[redacted]	[redacted]	[redacted]
5000	[redacted]	[redacted]	[redacted]
LD50 value: 4000 mg/kg bw (3300 – 5800 mg/kg bw)			
females			
730	[redacted]	[redacted]	[redacted]
950	[redacted]	[redacted]	[redacted]
1230	[redacted]	[redacted]	[redacted]
1600	[redacted]	[redacted]	[redacted]
2300	[redacted]	[redacted]	[redacted]
3000	[redacted]	[redacted]	[redacted]
3900	[redacted]	[redacted]	[redacted]
5000	[redacted]	[redacted]	[redacted]
LD_{50} value: 1700 mg/kg bw (1400-2200 mg/kg bw)			

* first number = number of dead animals
second number = number of animals with toxic signs
third number = number of animals used

Section A6.1.2 — 6.1 Acute Toxicity

Annex Point IIA6.1.2 — Acute dermal toxicity study on rat (Limit test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1991, HWG 1608 Technical – Acute dermal toxicity study on rats, [redacted], Report No [redacted], 1991-09-17 (unpublished)
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Methods used in this study are according to OECD-Guideline 402.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	Whitish powder.
3.1.2.2	Purity	[redacted] active substance.
3.1.2.3	Stability	Not reported.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Spargue-Dawley (Crj: CD, SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males + females
3.2.5	Age/weight at study initiation	Males: 7 weeks old; 229 –242 g Females: 7 weeks old; 157 –168 g
3.2.6	Number of animals per group	5 per sex/group
3.2.7	Control animals	No.
3.3	**Administration/ Exposure**	Dermal
3.3.1	Post-exposure period	14 days.

Section A6.1.2 — 6.1 Acute Toxicity

Annex Point IIA6.1.2 — Acute dermal toxicity study on rat (Limit test)

3.3.2	Area covered	4 × 5 cm
3.3.3	Occlusion	Semiocclusive
3.3.4	Concentration	2000 mg/kg bw
3.3.5	Vehicle	Polyethylene glycol 400
3.3.6	Concentration in vehicle	400 mg/ml
3.3.7	Total volume applied	5 ml/kg bw
3.3.8	Duration of exposure	24 h
3.3.9	Removal of test substance	Water
3.3.10	Controls	—
3.4	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	No effects.
4.2	**Pathology**	No effects.
4.3	**Other**	None.
4.4	**LD_{50}**	Give LD_{50} males + females: > 2000 mg/kg bw No lethal effect at maximal dose.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was done according to the OECD-Guideline 402. The acute dermal toxicity of tebuconazole was tested in Sprague-Dawley rats at the dermal limit dose of 2000 mg/kg bw. The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard when handling the substance.
5.2	**Results and discussion**	Neither death nor poisoning symptoms were found in the male and female rats after 24 hours exposure of the test substance at the dose level of 2000 mg/kg bw. No skin irritation findings were observed. Therefore, the dermal toxicity of tebuconazole is very weak.
5.3	**Conclusion**	
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Section A6.1.2 6.1 Acute Toxicity

Annex Point IIA6.1.2 Acute dermal toxicity study on rat (Limit test)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_1-1.2 Table for acute dermal toxicity in rats

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality (%)
males				
2000	[redacted]	[redacted]	[redacted]	[redacted]
LD_{50} value > 2000 mg/kg bw				
females				
2000	[redacted]	[redacted]	[redacted]	[redacted]
LD_{50} value > 2000 mg/kg bw				

*first number = number of dead animals

second number = number of animals with signs

third number = number of animals used

Section A6.1.3

Annex Point IIA6.1.3

6.1 Acute Toxicity

Acute inhalation toxicity to the rat (Limit test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], HWG 1608 – Study for acute inhalation to the rat, [redacted], Report No. [redacted], 1988-01-07
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD-Guideline 403
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	technical grade
3.1.2.1	Description	white powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The actual concentration of test substance was determined analytically by high performance liquid chromatography.
3.2	**Test Animals**	
3.2.1	Species	rat
3.2.2	Strain	Wistar (Bor:WISW)
3.2.3	Source	[redacted]
3.2.4	Sex	males and females (1:1)
3.2.5	Age/weight at study initiation	age: 8-12 weeks weight: 150-210 g
3.2.6	Number of animals per group	5 per sex per group
3.2.7	Control animals	Yes (air and vehicle)
3.3	**Administration/ Exposure**	Inhalation Toxicity was determined after aerosol or dust inhalation
3.3.1	Postexposure period	14 days

Section A6.1.3

Annex Point IIA6.1.3

6.1 Acute Toxicity

Acute inhalation toxicity to the rat (Limit test)

		Inhalation	
3.3.2	Concentrations	Nominal concentration	4000 mg/m³ (aerosol)
		Analytical concentration	371 mg/m³ (aerosol), 5093 mg/m³ (dust)
		both are maximum technically producible concentrations	
3.3.3	Particle size	Aerosol exposure:	
		MMAD (mass median aerodynamic diameter): 1.4 µm ± GSD (geometric standard deviation): 1.4 µm	
		percentage of particles ≤ 5 µm (respirability): 100 %	
3.3.4	Type or preparation of particles (dust study)	Dust exposure: (Preparation by an "Anderson-Impactor")	
		MMAD (mass median aerodynamic diameter): 12.8 µm ± GSD (geometric standard deviation): 1.9 µm	
		percentage of particles ≤ 5 µm (respirability): 8 %	
3.3.5	Type of exposure	Nose/head only	
3.3.6	Vehicle	ethanol / polyethylene glycol E 400 (1:1)	
3.3.7	Concentration in vehicle	20 % HWG 1608 (gram per volume)	
3.3.8	Duration of exposure	4 h	
3.3.9	Controls	controls were exposed to air or vehicle under the same conditions as the treatment groups	
3.4	**Examinations**	Clinical observations, necropsy	
3.5	**Method of determination of LC_{50}**	LC_{50} not determined	
3.6	**Further remarks**		
		4 **RESULTS AND DISCUSSION**	
4.1	**Clinical signs**	no effects	
4.2	**Pathology**	The rats sacrificed at the end of the observation period did not provide any indications of grossly apparent lung or other organ damage.	
4.3	**Other**		
4.4	**LC_{50}**	No lethal effect at the maximum concentration: LC_{50} > 371 mg/m³ (aerosol) and LC_{50} > 5093 mg/m³ (dust)	

Section A6.1.3

Annex Point IIA6.1.3

6.1 Acute Toxicity

Acute inhalation toxicity to the rat (Limit test)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was performed in compliance with OECD-Guideline 403. The type of exposure was head/nose under dynamic conditions. This study was carried out to support the results of a previous study for acute inhalation toxicity. As in this study no deaths occurred at analytical concentrations of 16 – 818 mg/m^3 (aerosol), only the maximum technically producible concentration was used. Additionally to aerosol exposure, the acute health hazard when handling the product was estimated by dust exposure.

5.2 Results and discussion

The study was performed at the maximum concentrations which could be obtained in the experimental design with respect to both aerosol and dust.

Neither lethality nor clinical effects were observed.

There were no indications of specific local lung toxicity or damage of organs at gross pathology.

5.3 Conclusion

The test substance showed an LC_{50} > 371 mg/m³ (aerosol) and an LC_{50} > 5093 mg/m³ (dust).

The study shows that the test substance has virtually no acute inhalation toxicity, either as aerosol (high inhalability) or as dust (lower inhalability).

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_1-1. Table for Acute Toxicity

Analytical concentration [mg/m^3]	Toxicological results*	Duration of clinical signs	Time of death
males			
air control	[redacted]	[redacted]	[redacted]
vehicle control	[redacted]	[redacted]	[redacted]
371 (aerosol)	[redacted]	[redacted]	[redacted]
5093 (dust)	[redacted]	[redacted]	[redacted]
LC_{50} value > 371 mg/m^3 (aerosol) or 5093 mg/m^3 (dust)			
females			
air control	[redacted]	[redacted]	[redacted]
vehicle control	[redacted]	[redacted]	[redacted]
371 (aerosol)	[redacted]	[redacted]	[redacted]
5093 (dust)	[redacted]	[redacted]	[redacted]
LC_{50} value > 371 mg/m^3 (aerosol) or 5093 mg/m^3 (dust)			

* first number = number of dead animals
second number = number of animals with toxic signs
third number = number of animals used

Section 6.1.4(01) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation toxicity in rabbits

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1988, Primary Eye Irritation of FOLICUR® (HWG 1608) Technical in Albino Rabbits, [redacted], Report No. [redacted], 1988-05-12 (unpublished)
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes 1) USA-EPA-FIFRA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, November 1984 (Section 158.135, 81-4); and 2) US-EPA-TSCA Health Effects Test Guidelines, September 1985 (Section 798.4500).
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	Tan crystalline powder
3.1.2.2	Purity	[redacted] active ingredient
3.1.2.3	Stability	Indefinitely at freezer conditions.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females (1:1)
3.2.5	Age/weight at study initiation	Age: young adult
3.2.6	Number of animals per group	3 males, 3 females
3.2.7	Control animals	Untreated eye served as internal control.

Section 6.1.4(01) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation toxicity in rabbits

3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	Test substance was used as delivered.
3.3.2	Amount of active substance instilled	0.1 grams
3.3.3	Exposure period	72 h, no rinse was performed after application of test substance.
3.3.4	Post exposure period	21 days
3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	Yes
3.4.1.1	Scoring system	Criteria for Evaluation of Ocular Lesions Cornea 0-4; opacity degree (area most dense taken for reading): [0=no ulceration or opacity, 1=scattered or diffuse areas of opacity (other than a slight dulling of the normal lustre), details of iris clearly visible; 2=easily discernible translucent area, details of iris slightly obscured; 3=nacreous area, no details of iris visible, size of pupil barely discernible; 4=opaque cornea, iris not discernible through the opacity] Iris 0-2; 0=normal; 1=folds markedly deepened, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive); 2=no reaction to light, haemorrhage, gross destruction (any or all of these) Conjunctiva Redness 0-3; 0=vessels normal; 1=some vessels definitely injected above normal; 2=diffuse, deeper crimson red, individual vessels not discernible; 3=diffuse beefy red Chemosis 0-4; 0=no swelling; 1=any swelling above normal (includes nictitating membrane); 2=obvious swelling with partial eversion of lids; 3=swelling with lids about half closed; 4=swelling with lids about half closed to completely closed Discharge 0-3; 0=no discharge; 1=any amount different from normal (does not include small amounts observed in inner canthus of normal animals); 2=discharge with moistening of the lids and hairs just adjacent to lids; 3=discharge with moistening of the lids and hairs and considerable area around the eye
3.4.1.2	Examination time points	60min, 24h, 48h, 72h Additional examinations occurred on days 7, 8, 14, and 21, in order to characterize the time-course and reversibility of lesions.
3.4.2	Other investigations	Examination of discharge
3.5	**Further remarks**	–

Section 6.1.4(01) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation toxicity in rabbits

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	–
4.2	**Average score**	Remark: the study bears no average scores. The following values were calculated only for this study summary using the documented individual scoring values
4.2.1	Cornea	Individual results:
		#203 male/#206 male/#208 male/#182 female/#183 female/#184 female
		24 h: 0/0/0/0/0/0
		48 h: 0/0/0/0/0/0
		72 h: 0/0/0/0/0/0
		Average score 24 h - 72 h: 0.00
4.2.2	Iris	Individual results:
		#203 male/#206 male/#208 male/#182 female/#183 female/#184 female
		24 h: 0/0/0/0/0/0
		48 h: 0/0/0/0/0/0
		72 h: 0/0/0/0/0/0
		Average score 24 h + 48 h + 72 h: 0.00
4.2.3	Conjunctiva	
4.2.3.1	Redness	Individual results:
		#203 male/#206 male/#208 male/#182 female/#183 female/#184 female
		24 h: 1/1/1/1/1/1
		48 h: 1/1/1/1/1/1
		72 h: 0/0/0/1/0/0
		Average score 24 h +48 h + 72 h: 0.72
4.2.3.2	Chemosis	Individual results:
		#203 male/#206 male/#208 male/#182 female/#183 female/#184 female
		24 h: 1/1/1/2/1/1
		48 h: 0/0/0/1/0/0
		72 h: 0/0/0/1/0/0
		Average score 24 h + 48 h + 72 h: 0.50
4.3	**Reversibility**	Yes
		Redness and chemosis of the conjunctiva occurred in all animals. Both effects were reversible in all animals within 8 days after application.
4.4	**Other**	Discharge of grade 2-3 was observed within 1-24 h after dosing. Effect resolved in five animals by 72 h after dosing and in all animals within 7 days after treatment.
4.5	**Overall result**	The test substance is a mild eye irritant causing transient (3-8 days) irritation to the conjunctiva.

Section 6.1.4(01) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation toxicity in rabbits

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted according to 1) USA-EPA-FIFRA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, November 1984 (Section 158.135, 81-4); and 2) US-EPA-TSCA Health Effects Test Guidelines, September 1985 (Section 798.4500). The report is also in compliance with OECD guideline 405.

The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard during handling of the substance.

5.2 Results and discussion

There were no signs of corneal opacities or lesions involving the iris in any animal during the study. All six rabbits developed redness (grade 1), chemosis (grade 1-2) and discharge (grade 2-3) of the conjunctiva 1-24 h after dosing. Chemosis and discharge had resolved in five animals by 72 h after dosing and in all animals by day 7. Redness had resolved in four rabbits by 72 hours after dosing and in the one remaining animal by day 7.

5.3 Conclusion

FOLICUR technical was tested in six New Zealand White rabbits for its potential to cause eye irritation. The test material caused discharge, redness and swelling of the conjunctiva that resolved by eight days after dosing. The test material did not cause corneal opacities or lesions of the iris. Based on these results, FOLICUR technical is a mild eye irritant that caused transient (3-8 days) irritation to the conjunctiva without causing lesions of the cornea or iris.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Appendix

Table A6_1_4E-1. Results of eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
Score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to 4
60 min	■	■	■	■
24 h	■	■	■	■
48 h	■	■	■	■
72 h	■	■	■	■
Average 24h + 48h + 72h	■	■	■	■
Reversibility*	▮	▮	▮	▮
Average time for reversion	▮	▮	■	■
* *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				

Section A6.1.4(02) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation study on rabbits.

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted], 1983, HWG 1608 – Study for acute toxicity, [redacted], Report No. [redacted], 1983-10-13 (unpublished) [redacted], 1990, HWG 1608 – c. n.: Tebuconazole (proposed) – Study for skin and eye irritation/corrosion in rabbits (Addendum to Report No. [redacted]), [redacted], Report No. [redacted], 1990-10-31 (unpublished)
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Study was performed according to the OECD-Guideline 405 and the EEC Directive 84/449/EEC.
2.2	**GLP**	[redacted] [redacted]
2.3	**Deviations**	[redacted]
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted] active substance
3.1.2.3	Stability	Stable within the study period.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White, HC:NZW
3.2.3	Source	[redacted]
3.2.4	Sex	Females.
3.2.5	Age/weight at study initiation	Adults, Weight: 2.9 – 3.2 kg
3.2.6	Number of animals per group	3

Section A6.1.4(02) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation study on rabbits.

3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Preparation of test substance	—
3.3.2	Amount of active substance instilled	100 µl (weight 50 mg) of test compound
3.3.3	Exposure period	24h
3.3.4	Post-exposure period	21 days
3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	Yes
3.4.1.1	Scoring system	Grades of ocular lesions:

Cornea 0 – 4

(0 = no finding, 1 = slight, disperse, diffuse opacification, 2 = extensive, diffuse opacification, iris blurred, 3 = mother-of-pearl-like opacification, iris and pupil hardly recognisable, 4 = complete opacification, ulceration)

Area of cornea opacity:

1 = ¼ or less, but not 0,
2 = more than ¼, but less than ½,
3 = more than ½, but less than ¾,
4 = more than ¾ up to complete surface

Iris 0 – 2

(0 = no finding, 1 = swelling, reddening, positive light reaction, 2 = severe reddening and swelling, no light reaction)

Conjunctival

Redness 0- 3

(0 = reddening, vessels normal, 1 = vessels abnormally filled, 2 = diffuse reddening, 3 = diffuse deep reddening)

Chemosis 0- 4

Swelling 0 – 4 (0 = no swelling, 1 = slight swelling, 2 = severe swelling, lids everted, 3 = lids cover one half of eye, 4 = lids cover more than half eye, necroses and ulcers on the conjunctivas)

Interpretation criteria:

Slight irritation:

	Irritation index:
Cornea opacity	1.00 to 1.99
Hyperaemia of iris, reaction to light	≥ 0.5
Erythema of conjunctivae	1.00 to 2.49
Chemosis	1.00 to 1.99

Changes persisting for more than 24 h, reversible within 7 d or less.

Section A6.1.4(02) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation study on rabbits.

Moderate irritation:

	Irritation index:
Cornea opacity	2.00 to 2.99
Hyperaemia of iris, reaction to light	1.00 to 1.50
- with 3 animals used	1.00 to 1.99
Erythema of conjunctivae	≥ 2.50
Chemosis	≥ 2.00

Injuries persisting for more than 24 h, reversible within 14 d or less.

Severe irritation: see moderate irritation, however reversible within 21 d or less

Corrosive:

	Irritation index:
Cornea opacity	≥ 3.0
Hyperaemia of iris, reaction to light	≥ 1.5
- with 3 animals used	= 2.0

or other significant tissue destruction (necrosis), that persist or are expected to persist for 21 days or more.

3.4.1.2	Examination time points	60min, 24h, 48h, 72h, 7d, 14d, 21 d
3.4.2	Other investigations	Dacryorrhoea (tear flow) Discharge 0 – 3 (0 = no discharge, 1 = slightly increased descharge swelling, 2 = discharge with slight moistening of periorbital areas, 3 = discharge with considerable moistening of periorbital areas)
3.5	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No effects.
4.2	**Average score**	
4.2.1	Cornea	Average score (24, 48, 72 h) for each animal tested: 0.0
4.2.2	Iris	Average score (24, 48, 72 h) for each animal tested: 0.0
4.2.3	Conjunctiva	
4.2.3.1	Redness	Average score (24, 48, 72 h): Animal 1: 0.3; animal 2: 0.0; animal 3: 0.0
4.2.3.2	Chemosis	Average score (24, 48, 72 h) for each animal tested: 0.0
4.3	**Reversibility**	Yes Reddening of conjunctiva in one animal, reversible at 48 hours.
4.4	**Other**	None.
4.5	**Overall result**	Not irritating to eyes.

Section A6.1.4(02) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute eye irritation study on rabbits.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The methods used in this study are in accordance with the OECD-guideline 405. This study for acute eye irritation toxicity in rabbits was conducted with the test substance tebuconazole. The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard when handling the substance.
5.2	**Results and discussion**	The study reveals that the product does not have a significant irritant potential on eye. The mild and transient reactions on the mucous membranes immediately following exposure of the test substance to the eye are considered as mechanically induced effects.
5.3	**Conclusion**	The results indicate that the tebuconazole may be regarded as not irritating to eye.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Appendix

Table A6_1_4E-1. Results of eye irritation study

	Cornea (area)	Iris	Conjunctiva		Discharge
Score (animal 1/animal 2/animal 3)	0 to 4#	0 to 2#	redness 0 to 3#	chemosis 0 to 4#	0 to 3#
60 min	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
24 h	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
48 h	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
72 h	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Average 24h, 48h, 72h	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Maximum average score (including area affected, max 110)	This scoring system was not applied.				
Reversibility*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Average time for reversion	—	—	48 h	24 h	24 h
* c : completely reversible n c : not completely reversible n : not reversible					

Grades of ocular lesions:

Cornea 0 – 4:

(0 = no finding, 1 = slight, disperse, diffuse opacification, 2 = extensive, diffuse opacification, iris blurred, 3 = mother-of-pearl-like opacification, iris and pupil hardly recognisable, 4 = complete opacification, ulceration)

Area of cornea opacity:
1 = ¼ or less, but not 0,
2 = more than ¼, but less than ½,
3 = more than ½, but less than ¾,
4 = more than ¾ up to complete surface

Iris 0 – 2:

(0 = no finding, 1 = swelling, reddening, positive light reaction, 2 = severe reddening and swelling, no light reaction)

Conjunctivas:

Redness 0 – 3:
(0 = reddening, vessels normal, 1 = vessels abnormally filled, 2 = diffuse reddening, 3 = diffuse deep reddening)

Swelling 0 – 4:

(0 = no swelling, 1 = slight swelling, 2 = severe swelling, lids everted, 3 = lids cover one half of eye, 4 = lids cover more than half eye, necroses and ulcers on the conjunctivas)

Discharge 0 – 3:

(0 = no discharge, 1 = slightly increased descharge swelling, 2 = discharge with slight moistening of periorbital areas, 3 = discharge with considerable moistening of periorbital areas)

Section A6.1.4(03) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute skin irritation study on rabbits.

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1983, HWG 1608 – Study for acute toxicity, [redacted], 1983-10-13 (unpublished) [redacted], 1990, HWG 1608 – c. n.: Tebuconazole (proposed) – Study for skin and eye irritation/corrosion in rabbits (Addendum to Report No. [redacted]), [redacted], Report No. [redacted], 1990-10-31 (unpublished)
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes Study was performed according to the OECD-Guideline 404 and the EEC Directive 84/449/EEC.
2.2	**GLP**	[redacted] [redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted] active substance
3.1.2.3	Stability	Stable within the study period.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White, HC:NZW
3.2.3	Source	[redacted]
3.2.4	Sex	Males + females.
3.2.5	Age/weight at study initiation	Adults, weight: 2.7 – 3.8 kg
3.2.6	Number of animals per group	3

Section A6.1.4(03) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute skin irritation study on rabbits.

3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
3.3.1.1	Preparation of test substance	Test substance was moistened with water.
3.3.1.2	Test site and Preparation of Test Site	Test site: dorso-lateral area of the trunk (treated skin area: 6cm^2 in size), Shaved skin, Exposed skin areas were washed with water.
3.3.2	Occlusion	Semi-occlusive.
3.3.3	Vehicle	—
3.3.4	Concentration in vehicle	—
3.3.5	Total volume applied	—
3.3.6	Removal of test substance	Water
3.3.7	Duration of exposure	24 h or other
3.3.8	Post-exposure period	14 days, 4 weeks or other
3.3.9	Controls	Untreated skin areas served as control.
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Dermal examination	Yes
3.4.2.1	Scoring system	Draize scores Reddening 0 – 4 (0 = no reddening, 1 = mild, 2 = minimal to moderate, 3 = moderate to severe reddening, 4 = deep reddening, with partial cauterisation) Swelling 0 – 4 (0 = no swelling, 1 = very slight, 2 = slight; margins well defined, 3 = moderate; margins raised approximately 1 mm, 4 = severe swelling; margins raised > 1 mm, swelling larger than the exposed area) Interpretation criteria: Irritation index: 0 – 0.99 No irritation 1.0 – 1.99 Slight irritation 2.0 – 2.99 Moderate irritation 3.0 - 4.0 Severe irritation
3.4.2.2	Examination time points	60min, 24h, 48h, 72h, 7d and 14d
3.4.3	Other examinations	None.

Section A6.1.4(03) 6.1 Acute toxicity

Annex Point IIA6.1.4 Acute skin irritation study on rabbits.

3.5	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Average score**	
4.1.1	Erythema	Average score (24 h, 48 h, 72 h) for each animals tested: 0.0
4.1.2	Oedema	Average score for each animals tested: 0.0
4.2	**Reversibility**	—
4.3	**Other examinations**	—
4.4	**Overall result**	0.0 (no irritation)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The methods used in this study are in accordance with the guidelines of the OECD-Guideline 404 and the EEC Directive 84/449/EEC. This study for acute dermal irritation toxicity in rabbits was conducted with the test substance tebuconazole. The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard when handling the test substance.
5.2	**Results and discussion**	The results indicate that the test substance may be regarded as not irritating to the skin.
5.3	**Conclusion**	
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_1-4S-1. Table for skin irritation study

Score (average animals investigated) (3 rabbits; exposure for 4 h under semi-occlusive conditions)	**Examination time point**	**Erythema** (reddening)	**Oedema** (swelling)
Average score Draize scores* (0 to maximum 4)	60 min	[redacted]	[redacted]
	24 h	[redacted]	[redacted]
	48 h	[redacted]	[redacted]
	72 h	[redacted]	[redacted]
Other times	7 d	[redacted]	[redacted]
Average score	24h, 48h, 72h	[redacted]	[redacted]
Reversibility:		[redacted]	[redacted]
Average time for reversibility		[redacted]	[redacted]

[#] Reddening (Erythema) 0 – 4

0 = no reddening,

1 = mild,

2 = minimal to moderate,

3 = moderate to severe reddening,

4 = deep reddening, with partial cauterisation

[#] Swelling (Oedema) 0 – 4

0 = no swelling,

1 = very slight,

2 = slight; margins well defined,

3 = moderate; margins raised approximately 1 mm,

4 = severe swelling; margins raised > 1 mm, swelling larger than the exposed area)

Section A6.1.5 — 6.1 Acute toxicity

Annex Point IIA6.1.5 — Skin sensitisation test in guinea pigs (Maximisation test method of Magnusson and Kligman)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1996, HWG 1608 – Study for the skin sensitization effect in guinea pigs (Guinea Pig Maximization Test according Magnusson and Kligman), [redacted], Report No: [redacted], 1996-11-20 (unpublished)
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes The methods used in this study are in accordance to the OECD-Guideline 406, the EC Guideline 92/69, method B.6 and the Pesticide Assessment Guidelines, subdivision F, § 81-6.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White solid
3.1.2.2	Purity	[redacted] of active substance
3.1.2.3	Stability	Stability of test material was ensured throughout the test period.
3.1.2.4	Preparation of test substance for application	a) <u>for induction:</u> tebuconazole was formulated in physiological saline solution containing 2% Cremophor EL b) <u>for challenge:</u> tebuconazole was formulated in physiological saline solution containing 2% (v/v) Cremophor EL
3.1.2.5	Pre-test performed on irritant effects	Yes

Section A6.1.5 — 6.1 Acute toxicity

Annex Point IIA6.1.5 — Skin sensitisation test in guinea pigs (Maximisation test method of Magnusson and Kligman)

3.2	**Test Animals**	
3.2.1	Species	Guinea pigs
3.2.2	Strain	Hsd Poc:DH (SPF-bred)
3.2.3	Source	[redacted]
3.2.4	Sex	
3.2.5	Age/weight at study initiation	Age: 5-7-weeks Weight: 306 –326 g
3.2.6	Number of animals per group	20 animals/group 10 animals/group served as control. 5 animals/groups served as range-finding group.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Study type: Adjuvant
3.3.1	Induction schedule	day 0 – day 7 see table in appendix
3.3.2	Way of Induction	Intradermal and topical Occlusive
3.3.3	Concentrations used for induction	Intradermal: 5% (= 0.05 g test substance/ml) Topical: 50% (= 0.5 g test substance/ml), (causing mild to moderate irritation)
3.3.4	Concentration Freund's Complete Adjuvant (FCA)	50 % (v/v) in physiological saline containing 2% Cremophor EL
3.3.5	Challenge schedule	day 21; see table in appendix
3.3.6	Concentrations used for challenge	40% (= 0.4 g test substance/ml) (usually maximum non-irritant concentration)
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	24h and 48h after challenge
3.3.9	Removal of the test substance	At the end of the exposure period (24 h), the remaining test substance was removed with physiological saline solution.
3.3.10	Positive control substance	—

Section A6.1.5 — 6.1 Acute toxicity

Annex Point IIA6.1.5 — Skin sensitisation test in guinea pigs (Maximisation test method of Magnusson and Kligman)

3.4	**Examinations**	
3.4.1	Pilot study	Yes (dose-range finding study for intradermal and topical induction and for challenge)
		Induction:
		[redacted]
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1	**Results of pilot studies**	[redacted]
4.2	**Results of test**	
4.2.1	24 h after challenge	Number of animals with signs of allergic reactions / number of animals: 0/20 test substance group 0/10 controls
4.2.2	48 h after challenge	Number of animals with signs of allergic reactions / number of animals: 0/20 test substance group 0/10 controls

Section A6.1.5 — 6.1 Acute toxicity

Annex Point IIA6.1.5 — Skin sensitisation test in guinea pigs (Maximisation test method of Magnusson and Kligman)

4.2.3	Other findings	None.
4.3	**Overall result**	Under the conditions of the maximisation test and with respect to the evaluation criteria the test substance thus exhibits no skin sensitisation.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The methods used to perform the study complied with the OECD-Guideline 406, the EC Guideline 92/69, method B.6 and the Pesticide Assessment Guidelines, subdivision F, § 81-6. The sensitivity and reliability of the Magnusson and Kligman methodology have been verified (using 2-mercaptobenzothiazole formulated with physiological NaCl containing 2% v/v Cremophor EL), and are monitored at regular intervals. The pertinent records are filed in the Bayer AG Toxicology archives. A study for skin sensitisation in guinea pigs was conducted with the test substance tebuconazole. The purpose of the study was to enable the product to be classified (labelling), and to assess the potential acute health hazard when handling the test substance.
5.2	**Results and discussion**	Appearance and behaviour of the test substance group were not different from the control group. By the end of the study the body weight development of the treatment group animals was in the same range as that of control group. 24 h and 48 h after the challenge treatment, skin reactions were neither detectable in treatment groups nor in controls.
5.3	**Conclusion**	Under the conditions of the maximisation test and with respect to the evaluation criteria the test substance thus exhibits no skin sensitisation.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_1_5-1. Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Inductions	Concentration of test substance in solution	Day of treatment	Application	Post-challenge observations*	
				24 h	48 h
Induction 1	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Induction 2	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Challenge	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

*first number = grade of reaction

(0 = no reaction, 1 = in places slight redness, 2 = moderate diffuse redness, 3 = intensive redness and swelling)

second number = number of animals with signs of allergic reactions

third number = number of animals in group

Table A6_1_5-2. Results of skin sensitisation test (incidence of skin reactions following challenge)

	Number of animals with signs of allergic reactions / number of animals in group			
	Control		Test group 40 % tebuconazole-solution	
	Test substance patch	Control patch	Test substance patch	Control patch
scored after 24h	[redacted]	[redacted]	[redacted]	[redacted]
scored after 48h	[redacted]	[redacted]	[redacted]	[redacted]

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Annex Point IIA6.2

6.2 Toxicokinetics

Absorption and elimination in rats

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], [Phenyl-U-^{14}C] HWG 1608: Study of biokinetic behaviour in the rat, [redacted] Report No. [redacted], 1987-10-06 [redacted], Addendum I - [Phenyl-U-^{14}C] HWG 1608: Study of biokinetic behaviour in the rat – Response to EPA requests and inquiries, [redacted], Report No [redacted], 1992-10-01
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EPA Pesticide Assessment Guidelines, Subdivision F §85-1
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	-
3.1.2.1	Description	-
3.1.2.2	Purity	[redacted] *
3.1.2.3	Stability	Administration of the test substance was carried out immediately after the preparation of the suspension in the vehicle. In this suspension the test substance was stable for at least 4 hours as tested by means of thin layer chromatography.
3.1.2.4	Radiolabelling	^{14}C, labelling in the benzene ring
3.2	**Test Animals**	
3.2.1	Species	rat
3.2.2	Strain	Wistar (BOR:WISW)
3.2.3	Source	[redacted]
3.2.4	Sex	males and females (1:1) for determination of plasma levels and excretion with urine and faeces Additional males for determination of excretion with expired air and for cholecystostomy.

Section A6.2(01) 6.2 Toxicokinetics

Annex Point IIA6.2 Absorption and elimination in rats

3.2.5	Age/weight at study initiation	weight: approximately 200 g
3.2.6	Number of animals per group	5 animals per group
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral / gavage
3.3.1	Preparation of test site	not applicable
3.3.2	Concentration of test substance	depending on dose; suspension of compound in 0.5 % aqueous tragacanth gel single dose: 2 or 20 mg/kg bw; in some groups pretreatment with 2 mg/kg bw of unlabelled test substance
3.3.3	Specific activity of test substance	84.4 µCi/mg
3.3.4	Volume applied	10 ml/kg bw
3.3.5	Size of test site	not applicable
3.3.6	Exposure period	not applicable
3.3.7	Sampling time	urine and bile: 1, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, 42 and 48 h faeces: 24 and 48 h plasma: 0.17, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 24, 32, 48, 56 and 72 h
3.3.8	Samples	see 3.3.7

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	not described
4.2	**Dermal irritation**	not applicable
4.3	**Recovery of labelled compound**	92.07 – 99.80 %
4.4	**Percutaneous absorption**	not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test substance was administered to male and female Wistar rats at oral doses of 2 and 20 mg/kg bw. In addition, rats of both sexes were first subjected to 14 days of treatment with a daily oral dose of 2 mg/kg of unlabelled test substance, followed by a single radioactive dose of 2 mg/kg 24 hours after the last of these doses. Furthermore, excretion of the radioactivity with the exhaled air (dose 20 mg/kg) and with the bile (dose 2 mg/kg) was studied in male rats.

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Annex Point IIA6.2

6.2 Toxicokinetics

Absorption and elimination in rats

5.2	**Results and discussion**	After oral administration the radioactivity of the test substance underwent largely quantitative elimination from the gastrointestinal tract, with a moderate to high absorption rate. The quantities of radioactivity excreted by the cholecystostomized animals with the bile and the urine were 90.9% and 7.4 %, respectively, of the recovered amount. At the time of sacrifice the radiolabelled residues in the body amounted to only 0.21 %. The small amounts of radioactivity determined in the faeces might be due to fractions already absorbed and secreted and/or diffused through the gastrointestinal mucosa. A figure of > 99 % was therefore obtained for the degree of absorption. Maximum relative concentrations (radioactivity measured per g tissue / radioactivity administered per g body weight) of 0.11 – 0.20 were reached in the plasma in the period from 0.33 to 1.7 hours after oral administration. Both the characteristics of the absorption rate and further characteristics based on analysis of the concentration course of the plasma radioactivity were sometimes significantly dependent upon the sex of the animal, the magnitude of the dose, and in a few cases also on the preliminary treatment. The radioactivity was rapidly eliminated from the animal organism: 3 days after oral administration radiolabelled residues of the order of 0.26 – 0.71 % of the recovered amount were found in the animal body excluding the gastrointestinal tract. Some 99 % of the total amount of radioactivity recovered was excreted within 3 days of oral administration of 2 or 20 mg/kg. The excretion was predominantly by the biliary/faecal route: some 65- 83 % was excreted with the faeces and only between 16 and 34 % with the urine. The excretion rate was found to be significantly dependent upon sex in all experimental groups, the females excreting about twice as much of the radioactivity with the urine as did the males. Male rats with bile fistula eliminated around 91 % of the recovered amount of radioactivity with the bile within 2 days of oral administration of 2 mg/kg. Part of the amount excreted primarily with the bile into the intestinal lumen was subject to enterohepatic recirculation in the intact (i.e. non-cholecystostomized) animals. Only a very small amount of radioactivity (0.032 %) was detected in the exhaled air within 3 days of oral administration of 20 mg/kg.	
5.3	**Conclusion**		
5.3.1	Reliability	[redacted]	*
5.3.2	Deficiencies	[redacted]	

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Annex Point IIA6.2

6.2 Toxicokinetics

Absorption and elimination in rats

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_2-1. Table (in vivo test)

Excretion of total radioactivity and radioactively labelled residues in the body 72 hours after administration (percent of the retrieved radioactivity)					
Biological material	**2 mg/kg**	**2 mg/kg pretreatment**	**20 mg/kg**	**2 mg/kg (bile fistula)**	**20 mg/kg (expired air)**
males					
bile				[redacted]	
(^{14}C)-carbondioxide					[redacted]
urine	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
faeces	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
body excl. GI-tract	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
GI-tract	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Factor of balance	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
females					
urine	[redacted]	[redacted]	[redacted]		
faeces	[redacted]	[redacted]	[redacted]		
body excl. GI-tract	[redacted]	[redacted]	[redacted]		
GI-tract	[redacted]	[redacted]	[redacted]		
Factor of balance	[redacted]	[redacted]	[redacted]		

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Annex Point IIA6.2

6.2 Toxicokinetics

Distribution in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], [Phenyl-UL-^{14}C] HWG 1608: Whole-body autoradiographic distribution of the radioactivity in the rat, [redacted] Report No. [redacted], 1988-03-25	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	HWG 1608	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	-	
3.1.2.1	Description	-	
3.1.2.2	Purity	[redacted]	*
3.1.2.3	Stability	The test substance was administered immediately after preparation of the administration solution. In the administration solution the test substance was stable for at least 4 hours as tested by thin-layer chromatography.	
3.1.2.4	Radiolabelling	^{14}C, labelling in the benzene ring	
3.2	**Test Animals**		
3.2.1	Species	rat	
3.2.2	Strain	Wistar (BOR:WISW)	
3.2.3	Source	[redacted]	
3.2.4	Sex	males	
3.2.5	Age/weight at study initiation	average weight: 197 g	
3.2.6	Number of animals per group	total of 7 animals: six were treated with radiolabelled test substance and one was treated with non-radioactively labelled test substance to check for chemographic effects of the parent compound as compared to the X-ray film emulsion.	
3.2.7	Control animals	Yes (see 3.2.6.)	

Section A6.2(02) 6.2 Toxicokinetics

Annex Point IIA6.2 Distribution in rats

3.3	**Administration/ Exposure**	Oral / gavage
3.3.1	Preparation of test site	not applicable
3.3.2	Concentration of test substance	2 mg/ml; suspension in 0.5 % tragacanth gel single dose of 20 mg/kg
3.3.3	Specific activity of test substance	84.4 µCi/mg
3.3.4	Volume applied	2 ml per animal
3.3.5	Size of test site	not applicable
3.3.6	Exposure period	not applicable
3.3.7	Sampling time	1, 4, 8, 24, 48 and 72 h (one animal was sacrificed at each time point)
3.3.8	Samples	whole-body autoradiography, blood samples
		4 RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	not described
4.2	**Dermal irritation**	not applicable
4.3	**Recovery of labelled compound**	not applicable
4.4	**Percutaneous absorption**	not applicable
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The radioactivity distribution of the test substance was studied in the rat by means of whole-body autoradiography for a period of 72 hours after administration. The substance was administered orally to male Wistar rats in a single dose of about 20 mg/kg.
5.2	**Results and discussion**	After oral administration the radioactivity of the test substance is absorbed from the intestinal tract of the rat obviously at a medium to high rate and almost completely, because 1 hour after administration radioactivity is detectable in all body tissues with the exception of the compact bone substance. One hour after administration, very high concentrations were discernible in the contents of the gastrointestinal tract, in the preputial gland, as well as in some areas of the mucosa of the nose, the tongue and in the epithelium of the oesophagus. High concentrations were limited to the liver, the cortex of the adrenal gland, the infraorbital gland, and to the hair follicles of the dorsal skin. Mean concentrations were found in all fat tissues, in the brain and spinal marrow, in the lung, the pancreas, the salivary glands, the heart, the testes and in the kidneys. Low to very low concentrations were present in the papilla of the kidneys, the musculature, the bone marrow, the thymus, and in the skin. Very low concentrations were found in the blood indicating a high speed of distribution of the radioactivity in the animal body after resorption. During the whole time of investigation, the ratio of the radioactivity

Section A6.2(02) **6.2 Toxicokinetics**

Annex Point IIA6.2 Distribution in rats

		concentrations among the tissues and organs showed only slight alterations. With increasing time after administration, the concentrations declined faster in the mucosa of the nasopharyngeal tract, in the fat tissues, the brain and spinal marrow as well as in the infraorbital gland, the preputial gland and in the hair follicles as compared to the mean body concentration. Due to temporarily increasing concentrations and/or to a slow elimination, the blood showed an intermediate level and the cortex of the adrenal gland a very high level of radioactivity at the end of the investigation. In addition, the evaluation of the autoradiographs alluded to a high biliary excretion combined with a long-lasting enterohepatic circulation of radioactivity as well as to a relatively slow renal elimination rate with a low fraction excreted.	
5.3	**Conclusion**		
5.3.1	Reliability	[redacted]	*
5.3.2	Deficiencies	[redacted]	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_2-1. Table for Percutaneous absorption (in vivo test)

NOT APPLICABLE!

	labelled compound	
	absolute amount	% of dose
Compound applied		100
Compartments with compound detected		
1. Protective appliances		
2. Liquid used for washing the skin		
3. Skin (with substance not removable)		
4. Blood		
5. Urine		
6. Faeces		
7. Removed organs *specify organs give sum*		
8. Remaining carcass		
9. Exhaled air		
Sum of #4 – 9: blood, excreta, removed organs, remaining carcass (= absorption)		
Sum of all detected labelled compound (#1 – 9) (=revovery)		

Section A6.2(03) 6.2 Toxicokinetics

Annex Point IIA6.2

Metabolism in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], FOLICUR: Metabolism part of the general metabolism study in the rat, [redacted], Report No. [redacted], 1987-12-21	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA §85-1	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Folicur, HWG 1608	
3.1.1	Lot/Batch number	-	
3.1.2	Specification	-	
3.1.2.1	Description	-	
3.1.2.2	Purity	[redacted]	*
3.1.2.3	Stability	The administration solution was stable for at least 4 hours.	
3.1.2.4	Radiolabelling	^{14}C phenyl-UL-^{14}C- and triazol-3,5-^{14}C-labelled compounds were used	
3.2	**Test Animals**		
3.2.1	Species	rat	
3.2.2	Strain	Wistar (BOR:WISW)	
3.2.3	Source	[redacted]	
3.2.4	Sex	males and females	
3.2.5	Age/weight at study initiation	average weight: 200 g	
3.2.6	Number of animals per group	five per sex per group	
3.2.7	Control animals	No	

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Annex Point IIA6.2

Metabolism in rats

3.3	**Administration/ Exposure**	Oral / gavage
3.3.1	Preparation of test site	not applicable
3.3.2	Concentration of test substance	0.2 or 2.0 mg/ml; suspension in 0.5 % tragacanth gel single dose: 2 or 20 mg/kg bw; in some groups pretreatment with 2 mg/kg bw of non-radioactive test substance
3.3.3	Specific activity of test substance	phenyl-UL-^{14}C-HWG 1608: 84.4µCi/mg triazol-3,5-^{14}C-HWG 1608: 56.5 µCi/mg
3.3.4	Volume applied	10 ml/kg bw
3.3.5	Size of test site	not applicable
3.3.6	Exposure period	not applicable
3.3.7	Sampling time	urine: 8, 24, 48 and 72 h faeces: 24, 48 and 72 h
3.3.8	Samples	urine, faeces, skin, gastrointestinal tract, carcass without gastrointestinal tract

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	not described
4.2	**Dermal irritation**	not applicable
4.3	**Recovery of labelled compound**	92 to 98 % of the administered radioactivity
4.4	**Percutaneous absorption**	not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The metabolism of the test substance after administration of either [phenyl-UL-^{14}C]-HWG 1608 or [triazol-3,5-^{14}C]-HWG 1608 to several groups of rats under varying experimental conditions was assayed. The dose groups were in accordance with the requirements of the EPA Assessment Guidelines §85-1: -single oral low dose of 2 mg/kg bw -14 daily single oral non-radioactive doses of 2 mg/kg, followed by a radioactive dose of 2 mg/kg on the 15th day -single oral high dose of 20 mg/kg In the main study the [phenyl-UL-^{14}C]-HWG 1608 was used. Each group consisted of 5 male and 5 female animals. In addition to these trials, the high dose of the triazole-labelled test substance was orally administered to both sexes. Purification and isolation of metabolites for identification and structure elucidation was done with samples of the excreta of rats.

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Annex Point IIA6.2

6.2 Toxicokinetics

Metabolism in rats

5.2 Results and discussion

There was no dose dependence detectable with the phenyl-labelled compound, but a significant dependence on the animals´ sex: Male animals excreted 15.5 to 17 % with the urine, female animals 26 to 35 %. Complementarily, the males showed a higher portion of excreted radioactivity in the faeces (77 to 80 %) as compared to females (60 to 67 %).

In the trials using the triazole-labelled compound no sex difference in the excretion pattern was observed, with the female animals´ pattern resembling that of the respective study with the phenyl-labelled compound.

Female animals preferably produced rather simple oxidation products like the hydroxy- and the carboxy-metabolites (HWG 2061 and HWG 2443, respectively) followed by conjugation and only minor cleavage of the triazole moiety.

Male animals exhibited a more complex metabolic behaviour by further oxidizing the "primary metabolites" to compounds of higher degrees of oxidation, like the "triol"- and the "keto-acid"-metabolites (ECW 4886 and ECW 4873, respectively) and additionally a cleavage of triazole as detected with the triazole-labelled compound.

In the investigations with the triazole-labelled fungicide free triazole was detected as a metabolite accounting for approximately 5 % in the urine of the male and 1.5 % in that of the female rats.

The identification rate in the trials with the phenyl-labelled compound – thus without taking triazole as a metabolite into account – reached 55 to 63 % in the balances of the male, and 70 to 76 % in those of the female animals.

5.3 Conclusion

5.3.1 Reliability [redacted] *

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_2-1. Table (in vivo test)

Comparison ot the metabolite profiles in the excreta 72 h after administration of phenyl-labelled test substance

Compound	2 mg/kg		Pre-treatment plus 2 mg/kg		20 mg/kg	
	male	female	male	female	male	female
HWG 2443	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ECW 4393 2/2	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ECW 4390	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
HWG 2061	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ECW 4873	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ECW 4908	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ECW 4886	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ECW 4882	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
HWG 2251	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
HWG 1608	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
sum identified	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

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Annex Point IIA6.2

6.2 Percutaneous absorption (in vivo test)

Dermal absorption study in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], Supplemental submission [redacted] – Revised report - Dermal absorption of ^{14}C-HWG 1608 technical in rats, [redacted], Report No. [redacted], 1991-04-08	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA §85-2	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	HWG 1608	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	technical grade	
3.1.2.1	Description	crystalline solid	*
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	The HWG 1608 was stable over 4 days at room temperature. The actual concentrations of the dosing solutions were 80 to 92 % percent of the nominal concentration.	
3.1.2.4	Radiolabelling	^{14}C	
3.2	**Test Animals**		
3.2.1	Species	rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	[redacted]	
3.2.4	Sex	males	
3.2.5	Age/weight at study initiation	adults weight: 183 to 239 g	
3.2.6	Number of animals per group	24 rats per dose group	
3.2.7	Control animals	No	

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Annex Point IIA6.2

6.2 Percutaneous absorption (in vivo test)

Dermal absorption study in rats

3.3	**Administration/ Exposure**	Dermal
3.3.1	Preparation of test site	The back of each animal was shaved with electric clippers and wiped with acetone to clean the skin approximately 24 hours prior to dosing.

3.3.2 Concentration of test substance

Dose groups (**mg/rat**)	Dose groups (**µg/cm²**)	Dose groups (**µg/cm²**)
nominal:	nominal:	actual:
0.01	0.67	0.604
0.1	6.7	5.86
1	67	52.4
10	670	547

3.3.3 Specific activity of test substance

Dose groups (mg/rat):	^{14}C-HWG 1608 (µCi):
0.01	2.67
0.1	28.1
1	39.5
10	39.5

3.3.4	Volume applied	0.25 ml/rat
3.3.5	Size of test site	15 cm²
3.3.6	Exposure period	24 hours
3.3.7	Sampling time	0.5, 1, 2, 4, 8 and 24h after initiation of skin contact
3.3.8	Samples	urine, faeces, blood, carcass, skin (application site)

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	no compound-related signs were observed during the course of study, no death occurred
4.2	**Dermal irritation**	dermal irritation not observed / described
4.3	**Recovery of labelled compound**	The mean recoveries for the various dose groups at the various time points ranged from 91 to 105 % with a tendency to lower recovery at the later time points.
4.4	**Percutaneous absorption**	By the direct method, approximately 53 to 58 %, and by the indirect method, approximately 59 to 65 % of the dose was absorbed in a 24-hour period. direct: mg absorbed = mg in (skin + carcass + blood + urine + faeces) indirect: mg absorbed = mg dose minus mg in (gauze patch + rubber ring rinse + application device rinse + skin wash)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was done according to FIFRA §85-2 Guideline. Male rats were treated dermally with 4 doses of radiolabelled test substance. At 0.5, 1, 2, 4, 8 and 24 h post-dosing, the amount of test substance was determined in urine, faeces, blood, carcass and skin. Additionally, the amount of unabsorbed test substance at the application site was determined.

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Annex Point IIA6.2

6.2 Percutaneous absorption (in vivo test)

Dermal absorption study in rats

5.2 Results and discussion

The actual doses of ^{14}C-HWG 1608 were 0.604, 5.86, 52.4 and 547 µg/cm^2, respectively, which were 78 to 90% of the nominal doses.

At the 0.604 to 52.4 µg/cm^2 dose levels the amount of test substance absorbed peaked at 0.5 to 1 hour after dosing and then remained fairly constant over the rest of the 24-hour exposure period. By the direct method, approximately 53 to 58 %, and by the indirect method, approximately 59 to 65 % of the dose was absorbed in a 24-hour period. The test substance was initially absorbed into the skin and then gradually migrated from the skin into the body with approximately 50 % of the absorbed dose entering the body by 24 hours post-dosing.

At the 547 µg/cm^2 dose, the test substance was rapidly absorbed into the skin (approximately 85% of the dose was absorbed at 0.5 hours post-dosing by direct and indirect methods) and then underwent a bidirectional flux from the skin with a decline in the total absorption being observed over the 24-hour exposure period (60% and 69% absorbed by direct and indirect methods, respectively, at 24 hours post-dosings. By 24 hours post-dosing, approximately 7 % of the dose absorbed at the 0.5 hour time point or 12 % of the dose absorbed at the 24 hour time point entered the body, suggesting a saturation of the transport mechanism in the skin at the high dose.

The rapid entry of the test substance into the skin was considered to be due to a solvent effect at all doses. The plateauing of absorption for the lower doses following the initial rapid influx into the skin indicates that the test substance is poorly absorbed via dermal exposure without the presence of ethanol or possibly some other organic solvent.

In the blood the maximum mean percentage of the applied dose was 0.061, 0.161, 0.112 and 0.017 for the 0.604, 5.86, 52.4 and 547 µg/cm^2 dose groups, respectively.

5.3 Conclusion

In conclusion, at the relevant doses of 0.604 to 52.4 µg/cm^2 approximately 50 % of the test substance was absorbed within eight hours with a maximum absorption of 58 % (direct method) or 65 % (indirect method) at 24 hours. Most of the dose was absorbed within the first hour of application

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

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

Table A6_2-1. Table for Percutaneous absorption (in vivo test)

Mean percentage of the dose of test substance in the skin and body at various time points DOSE (µg/cm²)								
Time	**0.604**		**5.86**		**52.4**		**547**	
(hour)	Skin	Body*	Skin	Body*	Skin	Body*	Skin	Body*
0.5	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
1	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
2	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
4	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
8	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
24	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

* body = blood + urine + faeces + carcass

Section A6.3.1 — 6.3 Short-term repeated dose toxicity (oral)

Annex Point IIA6.3 — Subacute oral toxicity study in rats

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1 REFERENCE

1.1 Reference

[redacted], 1984, HWG 1608 – Study of the subacute oral toxicity to rats, [redacted], Report No. [redacted], 1984-11-12 (unpublished)

[redacted], 1987, HWG 1608 – Study of the subacute oral toxicity to rats – Incidence tables of histopathological findings (Addendum to Report No. [redacted]), [redacted], Report No. [redacted], 1987-12-21 (unpublished)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No

Methods used in this study are comparable with the OECD-Guideline 407.

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

3.1 Test material As given in section 2 of dossier.

3.1.1 Lot/Batch number [redacted]

3.1.2 Specification As given in section 2 of dossier

Section A6.3.1 6.3 Short-term repeated dose toxicity (oral)

Annex Point IIA6.3 Subacute oral toxicity study in rats

3.1.2.1	Description	—
3.1.2.2	Purity	[redacted] of active substance
3.1.2.3	Stability	Prior to the start of the study, homogeneity and stability of the test compound formulations at the highest and lowest concentration over a storage period of 6 hours were analysed. Once during the study, the test compound concentration in the administration formulation (all test compound levels) was analysed.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar, Bor:WISW (SPF-bred)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Age: young adults (about 7 weeks) Weight: 146 –177 g
3.2.6	Number of animals per group	20 animals/sex/group Ten animals of each group served as recovery groups.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	Daily
3.3.3	Post-exposure period	4 weeks
3.3.3.1	Type	Gavage
3.3.3.2	Concentration	Gavage: 0, 30, 100 or 300 mg/kg bw
3.3.3.3	Vehicle	Aqueous suspension of 2% v/v Cremophor EL
3.3.3.4	Concentration in vehicle	0, 3, 10 or 30 mg/ml
3.3.3.5	Total volume applied	10 ml/kg bw
3.3.3.6	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, daily.
3.4.1.2	Mortality	Yes, daily.
3.4.2	Body weight	Yes, weekly and before necropsy.
3.4.3	Food consumption	No.

Section A6.3.1 6.3 Short-term repeated dose toxicity (oral)

Annex Point IIA6.3 Subacute oral toxicity study in rats

3.4.4	Water consumption	No.
3.4.5	Ophthalmoscopic examination	No.
3.4.6	Haematology	Yes, Number of animals: 5 animals/sex/group Time points: end of treatment period and end of recovery period Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, reticulocyte count, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV).
3.4.7	Clinical Chemistry	Yes, Number of animals: 5 animals/sex/group Time points: end of treatment period and end of recovery period Parameters: glucose, urea, total bilirubin, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase. Liver tissue: triglycerides, activities of cytochrome P-450, O-demethylase and N-demethylase.
3.4.8	Urinalysis	Yes Number of animals: 5 animals/sex/group Time points: end of treatment period and end of recovery period Parameters: sediment, pH, urobilinogen, protein, glucose, blood During urine collection (16 h) feed and water were withheld.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes Number of animals: 10 animals/sex/group Time points: end of treatment period and end of recovery period Organs: liver, kidneys, lungs, adrenals, gonads, thyroids, spleen, heart
3.5.2	Gross and histopathology	Yes 10 animals/sex/group for necropsy at the end of treatment period and end of recovery period 5 animals/sex of at least the control and high-dose group for histopathology at the end of treatment period and end of recovery period Organs: thyroid, heart, lung, lymph nodes, salivary glands, liver, spleen, kidneys, adrenals, testes, epididymides, ovaries, uterus, stomach, intestine (4 sites), bones (sternum and femur), musculature. In addition, two bone marrow smears (femur) were prepared.
3.5.3	Other examinations	Tests on the microsomal liver enzyme system; therefor pieces of organ tissue each weighing 0.8 – 1.2 g (0.1 – 0.2 g for triglycerides) were obtained.
3.5.4	Statistics	The quantitative results for individual animals were used to calculate arithmetic group means and standard deviations; the upper and lower confidence limits at the level of confidence of 1 – alpha = 95% and 1 – alpha = 99% were estimated. The results for the groups that received the test substance were compared with those for the control group using the significance test (U Test of Mann, Whitney and Wilcoxon) at the significance level of α = 5% and α = 1%.

Section A6.3.1 6.3 Short-term repeated dose toxicity (oral)

Annex Point IIA6.3 Subacute oral toxicity study in rats

3.6	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No effects up to and including 100 mg/kg bw. Dosing of 300 mg/kg bw resulted in mild lethargy. Polyuria occurred in few animals of the highest dose group during the 1st and 2nd week of treatment. Since this finding was not detected in the further course of the study and clinical chemistry and histopathology revealed no correlating findings, it is little toxicological relevance. No effects could be observed at the end of the post-treatment observation period.
4.1.2	Mortality	No treatment-related mortalities at any dose during exposure and post-treatment period.
4.2	**Body weight gain**	No effects up to and including 100 mg/kg bw. Dosing of 300 mg tebuconazole/kg bw resulted in a delayed body weight gain from week 1 until end of treatment (week 4). During the first week of the recovery period, body weight gain was compensated; all groups exhibited comparable values.
4.3	**Food consumption and compound intake**	—
4.4	**Ophtalmoscopic examination**	—
4.5	**Blood analysis**	
4.5.1	Haematology	No effects up to and including 30 mg/kg bw. Treatment with 100 and 300 mg tebuconazole/kg bw resulted in an effect on the red blood profile (haemoglobin concentration and haematocrit value were significant decreased; MCH and MCV were slightly slower in females). In the females of the 300 mg/kg dose group, a clearly higher leukocyte count in comparison to the control was observed. No effects could be observed at the end of the post-treatment observation period.
4.5.2	Clinical chemistry	No effects up to and including 30 mg/kg bw. At 300 mg tebuconazole/ kg bw an impairment of the liver function occurred (slight increase of alanine and aspartate aminotransferase in males and a marked increase of alkaline phosphatase, alanine and aspartate aminotransferase in females). Treatment with 300 mg tebuconazole/kg bw resulted in an induction of the examined microsomal enzyme systems (cytochrome P-450, O-demethylase, N-demethylase). Dosage of 100 mg/kg bw induced an increase of the demethylase activities, whereby only in males a statistically significance could be shown. The triglyceride concentration was elevated in high-dosed males. No effects could be observed at the end of the recovery period.
4.5.3	Urinalysis	No effects.

Section A6.3.1 6.3 Short-term repeated dose toxicity (oral)

Annex Point IIA6.3 Subacute oral toxicity study in rats

4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No effects up to and including 30 mg/kg bw. Elevated absolute and relative mean liver weights were found after dosing of 100 or 300 mg/kg bw. There were increases in the relative mean spleen weight for the male rats of the highest dose group and in the mean absolute and relative spleen weight for the female rats of the 100 and 300 mg/kg bw dose groups. The females of the 100 and 300 mg/kg bw dose groups showed an increased mean kidney weight. This finding was not obtained in males of the appropriate dose groups, but rather a decrease was observed, so that the finding in the case of the female animals can be regarded as having little relevance. Isolated statistically significant differences in mean weights were also calculated for a number of other organs (adrenals, testes, heart, lung). However, these were not dose-related and were probably caused by an unsymmetrical random distribution of the individual values within the populations to be compared. No treatment-related effects could be observed at the end of the recovery period.
4.6.2	Gross and histopathology	No effects during necropsy up to and including 300 mg/kg bw. No effects during histopathology up to and including 30 mg/kg bw. Dosing with 300 mg/kg bw revealed to increased fat in the liver of all animals. In addition the females exhibited an increase in periportal stroma, associated with bile duct proliferation. In the male rats, the centrilobular hepatocytes appeared enlarged. High dosed animals showed compound-related changes in the adrenal cortex, which indicates increased activity of the organ. In the females, sinus endothelial cells had proliferated and phagocytosed and increased fat vacuoles in the cells of zona fasciculata were found; an enlargement of the zone glomerulosa was found in the males. In the spleen of the same group, sclerosis of the red pulp, associated with sideropenia, was observed in both males and females. The sideropenia was also found in females of the 100 mg/kg bw dose group. The lungs of two high-dosed females indicated clefts in the structure of the cytoplasm of activated and proliferated endothelial cells. Two females of the same dose group exhibited an increased appearance of fat cells in the bone marrow, which resulted in a reduction in haematopoiesis in this tissue section. Animals of the recovery groups exhibited no microscopic organ changes, only high-dosed females showed an increase in fibre content in the red pulp of the spleen and in the periportal field of the liver. There were still fat vacuoles and mild reactions of sinus endothelial cells in the zona fasciculata. These findings indicate an incomplete recovery of the organ changes.
4.7	**Other**	—

Section A6.3.1 6.3 Short-term repeated dose toxicity (oral)

Annex Point IIA6.3 Subacute oral toxicity study in rats

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The objective of the study was to identify any potential toxic effects of tebuconazole, including the dose-response relationship in a mammalian species, and to establish a no-effect level. *

The methods used in this study are comparable with the OECD-Guideline 407. [redacted]

The administered dosages were selected on the basis of range-finding tests.

5.2 Results and discussion

The 4-week oral treatment of the male and female rats with 30 mg tebuconazole/kg bw was tolerated without observable effects.

Dosing of 300 mg/kg bw resulted in mild lethargy and a delayed body weight gain.

Treatment with 100 and 300 mg tebuconazole/kg bw resulted in an effect on the red blood profile. The increased leukocyte count of the same group could be explained by the activation of the haematopoietic system. Correlating with the mentioned results, there was an increase in absolute and relative spleens weights in the rats dosed with 100 mg/kg bw and above. The histopathological finding of sclerosis of the red pulp did not adequately explain the increase in spleen weight, because there was a tendency toward decreased spleen weights at the end of the observation period accompanied by the same pathological findings. It can thus be assumed that the increase in spleen weights is associated with the effect on the blood and with the increased metabolic efficiency of the spleen.

The clinical chemistry results revealed an impairment of the liver function in high-dosed animals. In the liver homogenate, an induction of the microsomal enzyme systems was found after treatment with 100 mg tebuconazole/kg bw and above. In addition, an increased triglyceride concentration in the liver tissue was detected in high-dosed males. Correlating with the mentioned results, there was an increase in absolute and relative liver weight in the rats from 100 mg/kg bw and above.

The clefts in the cytoplasmic structure of activated and proliferated endothelial cells of adrenals and lungs of female rats in the highest dose group indicate phagocytosis (occurrence of multinuclear giant cells).

Histopathological examinations showed that the liver, spleen and adrenals are the main targets of tebuconazole treatment.

Similar effects on liver, spleen and adrenals were also observable in high-dosed females at the end of the observation period. These findings indicate an incomplete recovery of the organ changes.

With the exception of the histopathological findings for females of the highest dose group, no biologically relevant changes in comparison with control were revealed by any of the tests and examinations performed on the animals sacrificed at the end of the observation period.

Overall, the test results show that the response of the female rats more sensitive to the oral administration of tebuconazole than that of the male rats.

Thus, a dose level of 30 mg tebuconazole/kg bw was tolerated by male

Section A6.3.1	6.3 Short-term repeated dose toxicity (oral)	
Annex Point IIA6.3	Subacute oral toxicity study in rats	
	and female rats with no observable effect when this dose was administered orally for 28 consecutive days.	
5.3	**Conclusion**	
5.3.1	LO(A)EL	Effects blood profile, impairment of liver function, sideropenia in the spleen; LOEL $_{\text{males + females}}$: 100 mg/kg bw
5.3.2	NO(A)EL	NOEL $_{\text{males + females}}$: 30 mg/kg bw
5.3.3	Other	—
5.3.4	Reliability	[redacted]
5.3.5	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_3-1.A Results of clinical chemistry, haematology and urinalysis

Due to results of the study, only results received at the end of the treatment period are presented.

Clinical chemistry	Sex	Unit	Control 0 mg/kg bw	Low dose 30 mg/kg bw	Medium dose 100 mg/kg bw	High dose 300 mg/kg bw
Alanine-aminotransferase		U/l				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Aspartat-aminotransferase		U/l				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Alkaline-phosphatase		U/l				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
O-demethylase		nmol/g/min				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
N-demethylase		nmol/g/min				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Cytochrome P-450		nmol/g/min				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Triglyceride		mcmol/g				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_3-1.A Results of clinical chemistry, haematology and urinalysis

Due to results of the study, only results received at the end of the treatment period are presented.

Haematology	Sex	Unit	Control 0 mg/kg bw	Low dose 30 mg/kg bw	Medium dose 100 mg/kg bw	High dose 300 mg/kg bw
Haemoglobin		G/l				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Haematocrit		L/l				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
MCH		Pg/e				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
MCV		Fl				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Leucocytes		Giga/l				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Reticulocytes		0/00				
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_3-2. Results of repeated dose toxicity study on rats (treatment group)

Parameter	Control 0 mg/kg bw		Low dose 30 mg/kg bw		Medium dose 100 mg/kg bw		High dose 300 mg/kg bw		Dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	10	10	10	10	10	10	10	10		
Mortality										
Clinical signs										
Body weight										
Clinical chemistry	Effects described in table A6_3-1.A Results of clinical chemistry, haematology and urinalysis									
Haematology										
Urinalysis										
Organ: liver										
Organ weight, relative and (absolute)										
Gross pathology										
Microscopic pathology										
Organ spleen										
Organ weight, relative (absolute)										
Gross pathology										
Microscopic pathology										

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_3-2. Results of repeated dose toxicity study on rats (treatment group), cont.

Parameter	Control 0 mg/kg bw		Low dose 30 mg/kg bw		Medium dose 100 mg/kg bw		High dose 300 mg/kg bw		Dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ adrenal										
Organ weight, relative (absolute)										
Gross pathology										
Microscopic pathology										
Organ: kidney										
Organ weight, relative (absolute)										
Gross pathology										
Microscopic pathology										
Organ: lung										
Organ weight										
Gross pathology										
Microscopic pathology										

[a] number of animals affected/total number of animals

↑ increase ↓ decrease — not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$