Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products

Evaluation of active substances

Document IIIA

Chlorocresol (CMK)

Product-types 1, 2, 3, 6, 9 and 13

FINAL CAR

April 2016

FRANCE

Section A1 Applicant

Annex Point IIA, I 1

1.1 Applicant

Company name: LANXESS Deutschland GmbH

Contact person 1: [redacted]

Address: Material Protection Products
Regulatory Affairs Business Line Actives & Disinfectants
[redacted]

Contact person 2: [redacted]

Address: Material Protection Products
Regulatory Affairs Business Line Actives & Disinfectants
[redacted]

1.2 Manufacturer of Active Substance (if different)

Manufacturer: LANXESS Deutschland GmbH
MPP-MF Alkylation Production Unit

Contact name: as Applicant

Location of manufacturing plant:
[redacted]

1.3 Manufacturer of Product(s) (if different)

Please refer to Document IIIB, Section 1

Section A1 Applicant

Annex Point IIA, I 1

	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 2013*
Materials and methods	
Conclusion	*A new address was notified:* *LANXESS Deutschland GmbH* [redacted]
Reliability	
Acceptability	
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 A2 **Subsection A2.4.3** **Annex Point IIA, II 2.4**	**Identity** OTHER NUMBERS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	[redacted]	
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	*09/09/08*	
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		

Section A2 **Subsection A2.8.5** **Annex Point IIA, II 2.8**	**Identity** OTHER NUMBERS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	[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	*09/09/08*	
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 A2 Identity of impurities and additives (active substance)

Subsection A2.8

Annex Point IIA, II 2.8

Official use only

This information is confidential and therefore provided separately in the confidential part of the dossier.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*17/03/09*
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	

Section A2 **Subsection A2.9** **Annex Point IIA, II 2.9**	**Identity** THE ORIGIN OF THE NATURAL ACTIVE SUBSTANCE OR THE PRECURSOR(S) OF THE ACTIVE SUBSTANCE	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	*09/09/08*	
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		

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

[redacted]

i) Description of process	The process description is provided in the confidential part of the dossier (A2.6 confidential).
ii) Workplace description	The whole reaction process is carried out in a closed device. All substance related occupational limit concentrations are met in the plant. Potential human exposure is only possible during loading and cleaning/service processes. All handling with respect on these processes are carried out using personal protection measures which are related to the respective task (up to full personal protection for special cleaning and service tasks where this is necessary).
iii) Inhalation exposure	Due to the effective personal protection during the above mentioned tasks and the closed plant technology including effective exhaustion neither dermal nor inhalation exposure is expected for the people involved in the production of CMK.
iv) Dermal exposure	Due to the effective personal protection during the above mentioned tasks and the closed plant technology including effective exhaustion neither dermal nor inhalation exposure is expected for the people involved in the production of CMK.

2.10.1.2 Intended use(s)

1. Professional Users

i) Description of application process	PT 1: 30-sec application onto hands followed by water rinse. PT 2: Surface disinfection by wiping with cloth PT 3: Surface disinfection by medium pressure spraying PT 6: Preparing pre-mix / pumping pre-mix into the solution to be preserved PT 13: Transferring OPP solid to pre-mix tank / Pumping OPP concentrate into the MWF circuit / Cutting metal using oil-based MWF, changing saw blades
ii) Workplace description	PT 1: Healthcare (hospital or medical practice) PT 2: Healthcare (hospital or medical practice) PT 3: Animal housing PT 6: Industrial facility PT 13: Metalworking shop

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

iii) Inhalation exposure	PT 1: 1.89×10^{-5} mg/kg bw/day PT 2: 0.0089 mg/kg bw/day PT 3: 0.044 mg/kg bw/day PT 6: 0.0472 mg/kg bw/day (all steps combined) PT 13: 0.01934 mg/kg bw/day (all steps combined)
iv) Dermal exposure	PT 1: 0.984 mg/kg bw/day PT 2: 0.305 mg/kg bw/day PT 3: 0.416 mg/kg bw/day PT 6: 0.121 mg/kg bw/day (all steps combined) PT 13: 0.3077 mg/kg bw/day (all steps combined)
2. Non-professional Users including the general public	
(i) via inhalational contact	PT 2: Surface cleaning: 1.2×10^{-4} mg/kg bw/day (all steps combined)
(ii) via skin contact	PT 2: Surface cleaning: 0.640 mg/kg bw/day (all steps combined) PT 6: Dishwashing: 0.0012 mg/kg bw/day
(iii) via drinking water	not relevant
(iv) via food	3.5×10^{-5} mg/kg bw/day (residues on plates, cutlery)
(v) indirect via environment	not relevant

2.10.2 Environmental exposure towards active substance

2.10.2.1 Production

(i) Releases into water	No direct contact of water with product is to be considered, as it is not part of the process. Water is used for cleaning purposes only, eg for the preparation of maintenance activities. The cleaning water is collected and released under controlled conditions to the central waste water treatment plant of the site. However, water is used for the scrubbing of waste air streams coming from the process. The scrubbing liquids contain, besides inorganic components, traces of organic matter - they are piped to the waste water treatment plant as well.
(ii) Releases into air	Waste air from the process is routed to an on site incineration unit and burned at about 850 degrees Celsius.
(iii) Waste disposal	The residue of the final purification step is collected, transported to the central incineration plant and burned under controlled conditions.

2.10.2.2 Intended use(s)

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

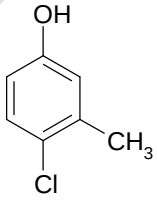
Affected compartment(s):	See Document II-B of dossier.
Water	See Document II-B of dossier.
Sediment	See Document II-B of dossier.
Air	See Document II-B of dossier.
Soil	See Document II-B of dossier.
Predicted concentration in the affected compartment(s)	See Document II-B of dossier.
Water	See Document 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.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*June 2013*
Materials and methods	
Conclusion	[redacted]
Reliability	
Acceptability	
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 A2 Identity of Active Substance

Annex point IIA, II 2

Official use only

Subsection (Annex Point)		
2.1	**Common name (IIA, II)**	Common name: p-Chloro-m-cresol EINECS Name: Chlorocresol Synonyms: CMK, PCMC Trade name: Preventol CMK
2.2	**Chemical name (IIA, II 2.2)**	IUPAC name: 4-Chloro-3-methylphenol CAS name: Phenol, 4-chloro-3-methyl-
2.3	**Manufacturer´s development code number(s) (IIA, II 2.3)**	Product number: 430587
2.4	**CAS No and EC numbers (IIA, II 2.4)**	
2.4.1	**CAS-No**	59-50-7
	Isomer 1	Not relevant
	Isomer n	Not relevant
2.4.2	**EC-No**	200-431-6
	Isomer 1	Not relevant
	Isomer n	Not relevant
2.4.3	**Other**	Not allocated
2.5	**Molecular and structural formula, molecular mass (IIA, II 2.5)**	
2.5.1	**Molecular formula**	C_7H_7ClO
2.5.2	**Structural formula**	
2.5.3	**Molecular mass**	142.6 g/mol
2.6	**Method of manufacture of the active substance (IIA, II 2.6)**	The method of manufacture of the active substance is confidential. This information is provided separately in the confidential part of the dossier.

Section A2 Identity of Active Substance

Annex point IIA, II 2

2.7	**Specification of the purity of the active substance, as appropriate (IIA, II 2.7)**	p-Chloro-m-cresol has a specified minimal purity of [redacted] Representative production batches of the active substance are analysed for their p-chloro-m-cresol content. This information is confidential and is provided separately in the confidential part of the dossier.
2.8	**Identity of impurities and additives, as appropriate (IIA, II 2.8)**	This information is confidential and therefore provided separately in the confidential part of the dossier.
2.8.1	**Isomeric composition**	Not relevant for the active substance.
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA, II 2.9)**	Not relevant as the active substance has no natural origin.

Section A2 Identity of Active Substance

Annex point IIA, II 2

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*31/03/08*
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 A3 **Subsection A3.8** **Annex Point IIIA, III 2**	**Physical and Chemical Properties of the Active Substance** STABILITY IN ORGANIC SOLVENTS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	*05/11/08*	
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		

Section A3 **Subsection A3.12** **Annex Point IIA, III 3.9**	**Physical and Chemical Properties of the Active Substance** FLASH POINT	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	05/11/08	
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		

Section A3 **Subsection A3.14** **Annex Point (-)**	**Physical and Chemical Properties of the Active Substance** VISCOSITY	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	05/11/08	
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		

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA, III 3.1)								
3.1.1 Melting point	EC method A.1 (DTA and ISTA)	Purity: [redacted] Specification: [redacted]	Exothermal decomposition of the active substance starts at 95 °C.	–	Y	[redacted]	Erstling, 2001a	x
	EC method A.1 (DTA)	Purity: [redacted] Specification: [redacted]	64.2 °C	–	Y	[redacted]	Erstling, 2007	
3.1.2 Boiling point	EC method A.2 (DTA)	Purity: [redacted] Specification: [redacted]	There is no boiling point up to the decomposition of the active substance.	Exothermal decomposition of the active substance starts at 95 °C.	Y	[redacted]	Erstling, 2001a	x
	EC method A.2	Purity: [redacted] Specification: [redacted]	242 °C	After boiling the liquid substance change the colour from colourless to yellowish. This is an indication for a beginning decomposition.	Y	[redacted]	Erstling, 2008	
3.1.3 Bulk density/ relative density								
Relative density	EC method A.3	Purity: [redacted] Specification: [redacted]	1.335 at 20 °C	–	Y	[redacted]	Erstling, 2001a	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Bulk density	ISO guideline 8967	Purity: [redacted] Specification: [redacted]	570 – 670 kg/m^3	10 lots of the active substance were investigated.	N	[redacted]	Haßmann, 1992	
3.2 Vapour pressure and Henry´s Law Constant (IIA, III 3.2)								
Vapour pressure	EC method A.4 (gas saturation method)	Purity: [redacted] Specification: [redacted]	7.75×10^{-02} Pa at 20 °C 1.44×10^{-01} Pa at 25 °C 2.29 Pa at 50 °C	–	Y	[redacted]	Olf, 2006a	x
Vapour pressure	EC method A.4	Purity: [redacted] Specification: [redacted]	1.4×10^{-03} Pa at 20 °C 6.0×10^{-03} Pa at 25 °C 3.8 Pa at 50 °C	–	Y	[redacted]	Wielpütz, 2008	
Henry's Law Constant	Calculation (Ratio between vapour pressure and water solubility)	–	Results at 20 °C: 3.35×10^{-03} Pa×m^3×mol^{-1} (pH 5) 3.25×10^{-03} Pa×m^3×mol^{-1} (pH 7) 2.70×10^{-03} Pa×m^3×mol^{-1} (pH 9)	–	N	[redacted]	Beiell, 2006	x
	Calculation based on QSAR methods using computer program from US EPA (EPIWIN software: HENRYWIN v3.10)	–	Results at 25 °C: 4.64×10^{-02} Pa×m^3×mol^{-1} (Bond method) 6.08×10^{-02} Pa×m^3×mol^{-1} (Group method)	–	N	[redacted]	Beiell, 2006	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
	Calculation (Ratio between vapour pressure and water solubility)	–	Results at 20 °C: 6.05×10^{-05} Pa×m^3×mol^{-1} (pH 5) 5.87×10^{-05} Pa×m^3×mol^{-1} (pH 7) 4.87×10^{-05} Pa×m^3×mol^{-1} (pH 9)	New calculation based on new vapour pressure study (Wielpütz, 2008)	–	[redacted]	–	x
3.3 Appearance (IIA, III 3.3)								
3.3.1 Physical state	Visual inspection	Purity: [redacted] Specification: [redacted]	Technical substance: solid pellets Purified substance: solid	–	N	[redacted]	Kraus, 2006a	
	CIPAC MT 171 (dustiness)	Batch No.: [redacted] Specification: [redacted]	Optical dust factor: 0.77 (nearly dust free)	–	Y	[redacted]	Güldner, 2009	x
3.3.2 Colour	Visual inspection	Purity: [redacted] Specification: [redacted]	Technical substance: nearly white Purified substance: nearly white	–	N	[redacted]	Kraus, 2006a	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.3 Odour	Olfactory inspection	Purity: [redacted] Specification: [redacted]	Technical substance: characteristic smell Purified substance: slight phenolic	–	N	[redacted]	Kraus, 2006a	
3.4 Absorption spectra (IIA, III 3.4)								
UV/VIS	The test was performed according to internal standard operation procedures.	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol was identified by UV/VIS spectrum; acetonitrile was used as solvent. Maxima at 228 nm (ε = 9625 l $mol^{-1}cm^{-1}$) and 281 nm (ε = 2241 l $mol^{-1}cm^{-1}$).	No UV absorbance above 290 nm.	N	[redacted]	Wesener, 2006	
IR	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol was identified by FTIR using a potassium bromide pellet.	–	N	[redacted]	Wesener, 2006	
NMR	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol was identified by ^{1}H-NMR spectrum and $CDCl_3$ was used as solvent.	–	N	[redacted]	Wesener, 2006	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
MS	Since no official (OECD) guideline is available the test was performed according to internal standard operation procedures.	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol was identified by 70 eV electron impact ionisation mass spectrum (EI-MS).	–	N	[redacted]	Wesener, 2006	
3.5 Solubility in water (IIA, III 3.5)	EC method A.6 (flask method)	Purity: [redacted] Specification: [redacted]	Results at pH 5: 2.5 g/L at 10°C 3.3 g/L at 20°C 4.5 g/L at 30°C Results at pH 7: 2.6 g/L at 10°C 3.4 g/L at 20°C 4.6 g/L at 30°C Results at pH 9: 3.1 g/L at 10°C 4.1 g/L at 20°C 5.5 g/L at 30°C	–	Y	[redacted]	Erstling, 2001b	
3.6 Dissociation constant (-)	OECD guideline 112	Purity: [redacted] Specification: [redacted]	pK = 9.4 ± 0.1 at 20 °C	The titration was performed in water as solvent.	Y	[redacted]	Reusche, 1991	x
	OECD guideline 112	Purity: [redacted] Specification: [redacted]	pK = 10.3 (measured) pK = 9.63 (calculated with ACD/pKa web service software)	The titration was performed in a mixture of 20 mL acetone and 40 mL water as solvent.	Y	[redacted]	Erstling 2001c	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
	Not applicable (statement)	–	The received pKa-value determined under study Reusche, 1991 (9.4) shows a sufficient correspondence to the calculated value under study Erstling, 2001c (9.63). Therefore for further purposes the pKa-value determined under study Reusche, 1991 is to be used.		N		Feldhues, 2006a	
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA, III 1)	CIPAC MT 157 and CIPAC MT 181	Purity: [redacted] Specification: [redacted]	n-Heptane: 4.9 g/L at 10 °C 8.5 g/L at 20 °C 15.4 g/L at 30 °C p-Xylene: 147.9 g/L at 10 °C 233.2 g/L at 20 °C > 250 g/L at 30 °C 1,2-Dichloroethane: 205.7 g/L at 10 °C > 250 g/L at 20 °C > 250 g/L at 30 °C The solubilities of CMK in 1-octanol, 2-propanol, acetone and ethyl acetate are > 250 g/L at each temperature.		Y	[redacted]	Jungheim, 2006a	
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA, III 2)	–	–	–	The active substance as manufactured does not include an organic solvent. Therefore no study regarding its stability in organic solvents was performed.	–	–	–	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.9 Partition coefficient n-octanol/water (IIA, III 3.6)	OECD guideline 107 (shake flask method)	Purity: [redacted] Specification: [redacted]	Log Pow = 3.02 at 22.0 ± 1 °C	–	Y	[redacted]	Reusche, 1991	x
	EC method A.8 (HPLC)	Purity: [redacted] Specification: [redacted]	Results at 25 °C: Log Pow = 2.0 (pH 5) Log Pow = 1.9 (pH 7) Log Pow = 1.3 (pH 9)	–	Y	[redacted]	Erstling, 2001c	x
	Calculation based on the 1-octanol and water solubility (Erstling, 2001b and Jungheim, 2006a)	Calculation	Log Pow = 1.97 (at 10 °C) Log Pow = 1.84 (at 20 °C) Log Pow = 1.72 (at 30 °C)	Only a slight temperature dependence could be observed, caused by the slight increase of the water solubility with increasing temperature.	Y	[redacted]	Jungheim, 2006b	x
	Not applicable (statement)	Not applicable (statement)	The studies Erstling, 2001c and Jungheim, 2006b were performed to demonstrate the pH and temperature dependence of the log Pow-value. The log Pow-value, determined according to the shake flask method (Reusche, 1991), is based on the exact determination of the concentration in both the water and octanol phase and is therefore, compared to the log Pow-values determined according to the HPLC method and the calculated log Pow-values, the more accurate value. The log Pow-value, determined in Reusche, 1991 is to be used exclusively.		N	[redacted]	Feldhues, 2007	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.10 Thermal stability, identity of relevant breakdown products (IIA, III 3.7)	EC method A.1 (DTA)	Purity: [redacted] Specification: [redacted]	Exothermal decomposition of the active substance starts at 95 °C.	–	Y	[redacted]	Erstling, 2001a	x
	OPPTS 830.6313 (comparable to CIPAC MT 46 / OECD 113)	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol is stable at normal and elevated temperatures (54 °C) over a 14-day period.	–	Y	[redacted]	Ambroz, 2000	
	Internal method 2011-0343801-92D (DTA/ISTA), corresponding to OECD guideline 102 / 113.	Purity: [redacted] Specification: [redacted]	The thermogram of the isothermal step analysis (ISTA) shows a slightly positive slope of the baseline starting at 95 °C. The slope increases significantly at approx. 240 °C. Therefore it can be concluded that the test item decomposes in a minor degree starting at 95 °C. A significant decomposition is observed at a temperature of approx. 240 °C.	–	Y	[redacted]	Königer, 2010 (Amendment to Erstling, 2001a)	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA, III 3.8)								
Flammability	EC method A.10	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol is not highly flammable.	–	Y	[redacted]	Heitkamp, 2006	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Evolution of flammable gases when contact with water	EC method A.12	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol does not liberate gases in hazardous amounts when contact with water.	–	Y	[redacted]	Heitkamp, 2006	
Pyrophoric properties	EC method A.13	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol does not deliver indications of pyrophoric properties during the realisation of other tests.	–	Y	[redacted]	Heitkamp, 2006	
Auto-flammability	EC method A.16	Purity: [redacted] Specification: [redacted]	p-Chloro-m-cresol does not undergo spontaneous combustion.	–	Y	[redacted]	Heitkamp, 2006	
3.12 Flash-point (IIA, III 3.9)	–	–	–	Not performed because the active substance is solid.	–	[redacted]	–	
3.13 Surface tension (IIA, III 3.10)	EC method A.5	Purity: [redacted] Specification: [redacted]	61.49 mN/m at 20 °C	CMK is not surface active.	Y	[redacted]	Olf, 2006b	x
3.14 Viscosity (-)	–	–	–	Not performed because the active substance is solid.	–	[redacted]	–	
3.15 Explosive properties (IIA, III 3.11)	–	–	Based on scientific judgement it is certified that due to the structural formula p-chloro-m-cresol contains no oxidising groups or other chemically instable functional groups. Thus the active substance is incapable of rapid decomposition with evolution of gases or release of heat, i.e. the solid material does not present any risk for explosion.		N	[redacted]	Kraus, 2006b	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.16 Oxidizing properties (IIA, III 3.12)	–	–	Based on scientific judgement it is certified that due to the structural formula p-chloro-m-cresol contains no oxidising groups in its molecular backbone and thus may not react exothermically with a combustible material. Therefore the active substance does not have oxidising properties.		N	[redacted]	Kraus, 2006c	
3.17 Reactivity towards container material (IIA, III 3.13)	Not relevant (statement based on experience in use)	Specification: [redacted]	Judged from the experience in use since many years CMK is not reactive towards the following container materials: paper, glass, PE, steel (zinc coated) and high-grade steel.	–	N	[redacted]	Kraus, 2006d	

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*09/02/2016*
Materials and methods	[redacted]

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

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

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*1.1.2 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	*1.1.3 Discuss if deviating from view of rapporteur member state*
Reliability	*1.1.4 Discuss if deviating from view of rapporteur member state*
Acceptability	*1.1.5 Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF PURE ACTIVE SUBSTANCE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

Official use only

1 REFERENCE

1.1	**Reference**	Jungheim, R., 2006c, Validation of a GC-Method for Preventol CMK (Pellets). Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany, Study No. 2006/0014/01 (unpublished), 2006-04-21
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The validation was performed according to SANCO/3030/99, rev. 4 of 11/07/00, guidance document of the European Commission for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II and Annex III of Directive 91/414.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	About 100 mg of test item is weighed into a GC-vial. After adding exactly 1 mL acetonitrile, the sample is dissolved by mechanical shaking. 1 µL of the solution is injected into the gas chromatograph and analysed according to the indicated conditions.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Gas chromatographic conditions:

Fused silica capillary column:

Stationary phase:	HP1 SE30
Length:	30 m
Internal diameter:	0.32 mm
Film thickness:	0.25 µm

Carrier gas:	helium
Split:	32 mL/min
Pressure:	60 kPa

Temperature program:

Temperature [°C]	Time [min]	Ramp [K/min]
80	--	4
130	--	20
280	1	--

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF PURE ACTIVE SUBSTANCE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

		Sample injection: split injection Injection volume: 1 µL Injector temperature: 250 °C
3.2.2	Detector	Flame ionisation detector (temperature: 300 °C).
3.2.3	Standard(s)	Analytical standard: CMK (Fa. Aldrich, product no. C5,540-2, Lot [redacted], purity: [redacted])
3.2.4	Interfering substance(s)	Substances of sample matrix may interfere.
3.3	**Linearity**	
3.3.1	Calibration range	To determine the linearity of the detector response determinations at three concentrations from 50 to 150% were performed.
3.3.2	Number of measurements	Each solution was analysed twice.
3.3.3	Linearity	Correlation coefficient: 0.9994. Equation of the calibration line: Y = 814.2532 * X – 1516.6709
3.4	**Specificity: interfering substances**	The identity of CMK was confirmed by comparison of the retention time of CMK in the test sample solution with the retention time obtained from CMK in the analytical standard solution. No interferences were observed.
3.5	**Recovery rates at different levels**	The accuracy of the method is established based on the findings for specificity, precision and linearity.
3.5.1	Relative standard deviation	The accuracy of the method is established based on the findings for specificity, precision and linearity.
3.6	**Limit of determination**	No limit of quantification or detection is given because the method is only used for checking the specification limits.
3.7	**Precision**	
3.7.1	Repeatability	Six sample determinations were performed. The relative standard deviation was 0.002%, which met the Horwitz requirements.
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF PURE ACTIVE SUBSTANCE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	An analytical method for the determination of CMK in the active substance as manufactured was developed and validated. CMK is separated by means of gas chromatography using flame ionisation detection (FID). The quantification is done by area normalisation with consideration of water content and unvolatisable components. The water content is determined by means of Karl Fischer titration where end point detection is performed biamperometrically using a Pt-indicator electrode. For the determination of unvolatisable components the residue of evaporation is determined. A test sample is slowly heated up under vacuum in a distilling oven until constant weight is reached.
4.2	**Conclusion**	The method has been completely validated for the active substance CMK by checking the parameters linearity, precision and specificity. GC experiments prove that CMK can be determined in the presence of impurities. All received validation data meet the requirements described in the Guidance document SANCO/3030/99 rev. 4 of 11/07/00. The method was found to be valid for the determination of CMK in the active substance as manufactured.
4.2.1	Reliability	Reliability indicator: [redacted]
4.2.2	Deficiencies	No

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1 ANALYTICAL METHOD FOR THE DETERMINATION OF PURE ACTIVE SUBSTANCE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	06/04/09
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	

Section A4.1 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.1

ANALYTICAL METHOD FOR THE DETERMINATION OF IMPURITIES IN THE ACTIVE SUBSTANCE AS MANUFACTURED

This information is confidential and provided separately in the confidential part of the dossier.

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*06/04/09*
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	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Official use only

1 REFERENCE

1.1	**Reference**	Brumhard, B, 2006, Analytical method 00998 for the determination of residues of Preventol CMK (4-chloro-3-methylphenol) in soil by HPLC-MS/MS. Bayer Crop Science AG, Development, Residues, Operator and Consumer Safety, Monheim am Rhein, Germany, Report No. MR-06/102 (unpublished), 2006-08-24
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 of March 17, 2004; Commission Directive 96/46/EC amending Council Directive 91/414/EEC of July 21, 1998; BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Soil samples of 20 g are extracted in a microwave extractor with 40 mL of a mixture of water/acetonitrile (7/3, v/v) for three minutes at 250 W. After extraction subsamples of extracts are centrifuged for 5 minutes at > 12000 RZB (g) to remove fine particles of the soil. 20 µL of final solution are injected into the HPLC instrument and analysed according to the indicated conditions.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Liquid chromatographic conditions:

Column:	LUNA 100A 5µ C18(2) – 50 mm x 2.0 mm; No. 00B-4252-B0; Phenomenex, Aschaffenburg, Germany
Injection volume:	20 µL
Oven temperature:	40 °C
Flow rate (column):	250 µL/min
Flow rate (interface):	250 µL/min
Mobile phases:	A: water/acetonitrile/acetic acid (900/100/1; v/v/v) B: acetonitrile/ acetic acid (1000/1; v/v)

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Isocratic pump for flushing the interface:
Mobile phases: milli-Q water/acetonitrile/acetic acid (500/500/0.1; v/v/v)
Flow rate (interface): 250 µL/min

Run time: 10 min
Retention time: approximately 3.5 min
HPLC gradient:

Time [min]	A [%]	B [%]
0	70	30
5	70	30
5.1	5	95
7.1	5	95
7.2	70	30
10	70	30

3.2.2	Detector	Mass spectrometric detector (MS/MS) with two Multiple Reaction Monitoring (MRM) transitions. For selection of the two MRM transitions the chlorine isotopic pattern of the parent molecule was used. The first MRM transition of CMK is the quantification precursor ion with the mass 141 [Cl 35] and the second MRM transition is the confirmatory precursor ion with the mass 143 [Cl 37].	x
3.2.3	Standard(s)	External standard (CMK, purity: [redacted])	
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.	
3.3	**Linearity**		
3.3.1	Calibration range	Solvent standard solutions and matrix-mixed standard solutions containing CMK were measured in a concentration range of 1 to 50 µg/L corresponding to a concentration in soil of 2 to 100 µg/kg. Six concentrations were measured for each test system.	
3.3.2	Number of measurements	Each concentration was measured twice.	
3.3.3	Linearity	For both mass transitions of CMK the detector showed linear correlation between concentration and peak area in the measured concentration range with correlation coefficients of 0.9986 to 0.9999.	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

3.4	**Specificity: interfering substances**	The blank values in all control samples were below 1.5 µg/kg (< 1/3 x LOQ), demonstrating that no background level of CMK was present in the test system. The MS/MS detection of CMK was slightly affected by the matrix. The peak area in soil Höfchen matrix solutions containing 1 to 50 µg/L CMK was reduced to approximately 90% of the corresponding peak area in solvent solutions for both mass transitions.
3.5	**Recovery rates at different levels**	Fortified specimens of two different soil types (Höfchen and Laacher Hof) were analysed five times for each fortification level. The fortification levels were 5 and 50 µg/kg. The overall mean recovery of the method was 96% for the quantification ion and 98% for the confirmatory ion. Recovery rates determined for each soil type, fortification level and mass transition are shown detailed in Table A4_2-1 and Table A4_2-2.
3.5.1	Relative standard deviation	The overall relative standard deviation for the quantification ion of CMK was 6.1% and for the confirmatory ion 4.1%. Relative standard deviations for each soil type, fortification level and mass transition are shown detailed in Table A4_2-1 and Table A4_2-2.
3.6	**Limit of determination**	The limit of quantification is 5 µg/kg and the limit of detection 1.5 µg/kg.
3.7	**Precision**	
3.7.1	Repeatability	The precision was determined from the recovery rates. The overall relative standard deviation for the quantification ion of CMK was 6.1% and for the confirmatory ion 4.1%. Details are presented in Table A4_2-1 and Table A4_2-2.
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	CMK was extracted from soil samples using a water/acetonitrile mixture. Subsamples of extracts are centrifuged to remove fine particles of the soil. Identification and quantitative determination of the active substance are done by HPLC using electrospray MS/MS-detection with two Multiple Reaction Monitoring (MRM) transitions.
4.2	**Conclusion**	A method for the determination of CMK residues in soil was developed and validated successfully according to SANCO/825/00 rev.7. Because of the high selectivity of the HPLC-MS/MS method and validation of two MRM transitions, an additional confirmatory method is not required.
4.2.1	Reliability	Reliability indicator: [redacted]
4.2.2	Deficiencies	No

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*09/02/2016*
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 A4_2-1: Recovery rates of CMK in soil (Quantification ion)

Fortification level [µg/kg]	Soil type	Single values [%]					Mean value [%]	Relative standard deviation [%]
5	Höfchen	95	103	93	100	106	99	5.6
5	Laacher Hof	90	94	96	93	99	94	3.3
Mean of all 5 µg/kg single values							97	5.2
50	Höfchen	103	103	102	98	103	102	2.1
50	Laacher Hof	89	90	89	93	87	90	2.4
Mean of all 50 µg/kg single values							96	7.2
Mean of all Höfchen values							*101*	*4.2*
Mean of all Laacher Hof values							*92*	*3.9*
Grand mean							**96**	**6.1**

Table A4_2-2: Recovery rates of CMK in soil (Confirmatory ion)

Fortification level [µg/kg]	Soil type	Single values [%]					Mean value [%]	Relative standard deviation [%]
5	Höfchen	105	95	102	102	101	101	3.4
5	Laacher Hof	101	91	98	96	100	97	4.0
Mean of all 5 µg/kg single values							99	4.1
50	Höfchen	102	100	100	102	104	102	1.5
50	Laacher Hof	95	94	94	94	93	94	0.9
Mean of all 50 µg/kg single values							98	4.3
Mean of all Höfchen values							*101*	*2.5*
Mean of all Laacher Hof values							*95*	*3.3*
Grand mean							**98**	**4.1**

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Official use only

1 REFERENCE

1.1	**Reference**	Feldhues, E., 2006b, Validation of an analytical method for the determination of Preventol CMK in air samples. Bayer Industry Services, BIS-SUA-Analytics, Leverkusen, Germany, Report No. 2006/0014/03 (unpublished), 2006-08-30
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The validation was performed according to SANCO/3029/99 rev. 4 of 11/07/00, Residues guidance document of the European Commission for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II and Annex III of Directive 91/414.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	With an expected amount of 1 µg/m^3 200 L air are pumped through a Tenax tube using a Du-Pont-pump. With higher expected amounts in the air a reduced air volume is sampled. The content of employed Tenax tube is converted into a 10 mL beaded rim bottle. Exactly 2 mL ethanol are added, the bottle is closed and shaken for 30 min. After filtration 3 µL of the final solution are injected into the GC and analysed according to the indicated conditions.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Chromatographic conditions:

column: Fused silica capillary

Stationary phase:	DB 1701
Length:	60 m
Internal diameter:	0.32 mm
Film thickness:	0.25 µm
Carrier gas:	helium
Pressure:	80 kPa
Carrier gas stream:	2 mL constant flow
Sample injection:	Pulsed splitless injection
Injection volume:	3 µL

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

		Temperatures: Injection temperature: 250 °C MS source: 230 °C Quadrupole: 150 °C Transfer-Line: 280 °C Temperature program: Temp [°C] / Time [min] / Ramp [°C/min] 100 / -- / 10 260 / 4 / -- Retention time: 10.3 min
3.2.2	Detector	Mass spectrometric detector in Selected Ion Monitoring (SIM) mode. Ionisation: Electron impact EM Voltage: 2400 EMV Mass range (SIM-Mass): Target ion: 142.10 m/z Qualifier 1: 144.10 m/z Qualifier 2: 107.10 m/z Qualifier 3: 77.10 m/z
3.2.3	Standard(s)	External standard (CMK, purity: [redacted])
3.2.4	Interfering substance(s)	Substances of sample matrix or adsorption material may interfere with CMK.
3.3	**Linearity**	
3.3.1	Calibration range	To determine the linearity of the detector response, determinations at seven concentrations, covering a range between 0.1 and 5 µg/m^3 (corresponding to test solution concentrations between 10 and 500 µg/L) were tested. For higher expected amounts in the air a reduced air volume has to be sampled. The single test solution concentrations used were: 9.2, 22.9, 54.9, 91.5, 183.0, 457.6 and 549.1 µg/L.
3.3.2	Number of measurements	Each concentration was measured twice.
3.3.3	Linearity	The correlation coefficient was 0.9965.

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

3.4	**Specificity: interfering substances**	Identification of active substance was performed by GC-MS of a solution of the test substance CMK, Batch no. [redacted], after extracting it from Tenax tube. The mass spectrum with the following typical mass fragment ions: m/z = 142.1, m/z = 144.1, m/z = 107.1 and m/z = 77.1 was identified as CMK by comparison with the mass spectrum of CMK given in the mass spectra library. The quantification was performed by monitoring all four target masses of the component.	
3.5	**Recovery rates at different levels**	For determination of precision and accuracy six Tenax tubes were each fortified with 6 µL of a stock solution containing 0.0626 µg CMK and six Tenax tubes were each fortified with 20 µL of a stock solution containing 0.209 µg CMK. 200 L air were pumped through each Tenax tube (nominal concentration 0.313 and 1.045 µg/m^3). The mean recovery was 76.33% (n = 6) at a nominal concentration of 31.3 µg/L and 77.03% (n = 6) at a nominal concentration of 104.5 µg/L, respectively. The overall mean recovery was 76.68% (n = 12). Recovery results are shown detailed in Table 4_2-1. The received mean recovery values were in the range of 70 – 100% and meet the requirements of the Guidance document SANCO/3029/99 rev. 4. Additionally the efficiency of extraction and the retention efficiency of the sorbent material were investigated. With a recovery rate of 92.8% for the first of three extractions, a satisfactory efficiency of extraction was reached and with a recovery rate of 79.7% the retention efficiency of the sorbent material is considered as sufficient.	
3.5.1	Relative standard deviation	The relative standard deviation was 8.95% at a nominal concentration of 31.3 µg/L and 6.96% at a nominal concentration of 104.5 µg/L, respectively. The overall relative standard deviation was 7.65% (n = 12).	
3.6	**Limit of determination**	The limit of quantification is 0.1 µg/m^3 air.	x
3.7	**Precision**		
3.7.1	Repeatability	Please refer to point 3.5 (recovery rates).	
3.7.2	Independent laboratory validation	No independent laboratory validation is available.	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	With a Du-Pont-pump a defined air volume is aspirated to a Tenax adsorption tube. The adsorpted CMK is extracted from the tube with ethanol. The amount of CMK in the extraction solvent is determined by means of gas chromatography using mass spectroscopic detection in the selected ion monitoring mode. Quantification is performed by the external standard method.
4.2	**Conclusion**	The method has been completely validated on CMK by checking the parameters linearity, specificity, precision, accuracy, the efficiency of extraction, the retention efficiency of the sorbent material as well as the limit of quantification. All received validation data meet the requirements described in the Guidance document SANCO/3029/99 rev. 4 of 11/07/00. The method was found to be valid.
4.2.1	Reliability	Reliability indicator: [redacted]
4.2.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*09/02/2016*
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_2-1: Results of recoveries

Nominal concentration [µg/L]	Recovery rate [%]		Mean recovery (n=6) [%]	Relative standard deviation [%]	Overall mean recovery (n=12) [%]	Overall relative standard deviation [%]
31.3	72.204	78.435	76.33	8.95	76.68	7.65
	66.454	86.901				
	76.038	77.955				
104.5	72.249	78.469	77.03	6.96		
	79.426	79.904				
	83.254	68.900				

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Official use only

1 REFERENCE

1.1	**Reference**	Krebber, R., 2006, Analytical method 01004 for the determination of Preventol CMK (4-chloro-3-methylphenol) in drinking and surface water by HPLC-MS/MS. Bayer Crop Science AG, Development, Residues, Operator and Consumer Safety, Monheim am Rhein, Germany, Report No. MR-06/112 (unpublished), 2006-09-05
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 of March 17, 2004; Commission Directive 96/46/EC amending Council Directive 91/414/EEC of 16 July 1996; BBA-Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Water samples are directly injected into the HPLC-MS/MS instrument.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Liquid chromatographic conditions:

Pre-column:	Aqua C18, 4 x 3 mm, article no. AJO-7511, Phenomenex, Aschaffenburg, Germany
Column:	Luna 5µ C18(2) 100A, 150 x 2 mm; article no. 00F-4252-B0, Phenomenex, Aschaffenburg, Germany
Particle size:	5 µm
Injection volume:	250 µL
Oven temperature:	40 °C
Mobile phases:	A: Milli-Q-water / acetonitrile / acetic acid (900/100/0.1; v/v/v) B: acetonitrile / acetic acid (1000/0.1; v/v)
Run time:	15 min
Flow rate:	0.4 mL/min
Retention time:	approx. 6.8 min

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

		Gradient: Time [min] / A [%] / B [%] 0 / 80 / 20 1 / 80 / 20 10 / 5 / 95 12 / 5 / 95 12.1 / 80 / 20 15 / 80 / 20	
3.2.2	Detector	Mass spectrometric detector (MS/MS) with two Multiple Reaction Monitoring (MRM) transitions. For selection of the two MRM transitions the chlorine isotopic pattern of the parent molecule was used. The first MRM transition of CMK is the quantification precursor ion with the mass 141 [Cl 35] and the second MRM transition is the confirmatory precursor ion with the mass 143 [Cl 37].	x
3.2.3	Standard(s)	External standard (CMK, purity: [redacted])	
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.	
3.3	**Linearity**		
3.3.1	Calibration range	The linearity of HPLC-MS/MS detection was determined using seven concentrations of CMK in surface water in the range of about 0.04 µg/L to 10.01 µg/L with two MRM transitions.	
3.3.2	Number of measurements	Each concentration was measured at least twice.	
3.3.3	Linearity	The correlation coefficient was 0.9995 for both MRM transitions.	
3.4	**Specificity: interfering substances**	The MS/MS detection of CMK was slightly affected by the matrix. The peak areas of both MRM transitions in a surface water sample containing 1 µg/L show a slight difference to the corresponding peak areas in deionised water. No residues of CMK were detected in the surface water control samples.	
3.5	**Recovery rates at different levels**	Because of the direct measurement of fortified samples without separate extraction and clean-up steps it is not possible to determine recovery rates and therefore, an estimate of accuracy of the analytical technique was made by an assessment of the linearity of matrix calibration and by determination of the reproducibility of sample analysis. However, for additional demonstration of the reliability of the method, validation samples (please refer to point 3.7.1 (Precision – Repeatability)) were evaluated like recovery rates using calibration curves. Results (percentage found, mean recoveries and relative standard deviations) are shown detailed in Table A4_2-1 and Table A4_2-2. The mean percentages found were between 98% and 102% for both fortification levels and MRM transitions. The corresponding relative standard deviations were between 1.7% and 4.1%.	
3.5.1	Relative standard deviation	Please refer to point 3.5 (Recovery rates).	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

3.6	**Limit of determination**	The limit of quantification for CMK is 0.05 µg/L for both MRM transitions. The limit of detection for both MRM transitions was determined to be 0.02 µg/L.
3.7	**Precision**	
3.7.1	Repeatability	Surface water samples were fortified with CMK at 0.05 µg/L and at 0.5 µg/L. These test solutions were injected ten times each into the HPLC-MS/MS instrument. The relative standard deviation for the peak area of the quantification ion of CMK was 3.9% (0.05 µg/L) and 2.0% (0.5 µg/L), for the confirmatory ion 2.2% (0.05 µg/L) and 1.9% (0.5 µg/L), respectively. The relative standard deviation for the retention time was ≤ 0.1% for both fortification levels and both MRM transitions.
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	The method describes the determination of CMK residues in drinking and surface water by HPLC-MS/MS. Water samples are directly injected into the HPLC instrument. Identification and quantitative determination are done by means of electrospray MS/MS-detection with two Multiple Reaction Monitoring (MRM) transitions.
4.2	**Conclusion**	A method for the determination of CMK residues in surface and drinking water was developed and validated successfully according to SANCO/825/00 rev. 7. A validation for drinking water was not necessary because the limit of quantification for surface water is below the drinking water limit of 0.1 µg/L. Because of the high selectivity of the HPLC-MS/MS method, an additional confirmatory method is not required.
4.2.1	Reliability	Reliability indicator: [redacted]
4.2.2	Deficiencies	No

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*09/02/2016*
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_2-1: Percentages of CMK (quantification ion) found in fortified surface water samples

Sample concentration	Percentage found						
µg/L	Sample values [%]					Mean [%]	RSD [%]*
0.05	103	103	98	102	94	98	4.1
	96	95	98	93	93		
0.5	99	104	101	99	104	101	2.0
	101	103	99	101	103		

*RSD = Relative standard deviation

Table A4_2-2: Percentages of CMK (confirmatory ion) found in fortified surface water samples

Sample concentration	Percentage found						
µg/L	Sample values [%]					Mean [%]	RSD [%]*
0.05	104	99	99	102	102	101	2.5
	100	96	102	103	98		
0.5	103	102	102	99	105	102	1.7
	100	101	100	103	101		

Section A4.2 **Annex Point IIA, IV 4.2**	**Analytical Methods for Detection and Identification** ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN 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:	[redacted]	
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	*07/10/08*	
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		

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products (01)

Annex Point IIA7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Erstling, K. and Feldhues, E. (2001): Abiotic degradation Bayer AG, ZF – Zentrale Analytik, Leverkusen, Germany, Report No. A 01/0108/04 LEV, unpublished, Date: 2001-08-31; amended: 2007-02-22 Knopf (2001): Analytical characterisation Bayer AG, ZF – Zentrale Analytik, Leverkusen, Germany, Report No. A 01/0108/00 UER, unpublished, Date: 2001-08-29	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EC Guideline 92/69/EC, C.7	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Non-radiolabelled test substance	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	-	
3.2	**Reference substance**	Not reported	
3.2.1	Initial concentration of reference substance	Not reported	
3.3	**Test solution**	The study was carried out with buffer solutions at three pH levels: - pH 4: citric acid/ sodium hydroxide/sodium chloride buffer - pH 7: potassium dihydrogen phosphate/di-sodium hydrogen phosphate buffer - pH 9: borax/hydrochloric acid All buffers were ready to use solutions (see Table A7_1_1_1_1-1). The description of the test solution is given in Table A7_1_1_1_1-2.	X

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products (01)

Annex Point IIA7.6.2.1

3.4	**Testing procedure**	
3.4.1	Test system	The test system is described in Table A7_1_1_1_1-3.
3.4.2	Temperature	50°C:
3.4.3	pH	4 / 7 / 9
3.4.4	Duration of the test	5 days
3.4.5	Number of replicates	For each pH value five determinations were performed during the test, corresponding to the sampling dates.
3.4.6	Sampling	The sampling intervals were: 0, 1, 24, 96 and 120 hours (5 days).
3.4.7	Analytical methods	All test solutions were analysed by HPLC without further dilution. Quantification was performed by area normalisation, the detected area units at time t = 0 were set to 100 %. The concentrations of p-chloro-m-cresol were determined using reversed phase HPLC under the following conditions: Column: LiChrospher RP 8, 5 µm, 125 x 4 mm; Mobile phase: 400 ml acetonitrile/ 600 ml water + 1 ml phosphoric acid (85%) ; Flow rate: 1.5 ml/min. Column temperature: 40°C Detector: UV, 200 nm Injection volume: 5 µl
3.5	**Preliminary test**	No

4 RESULTS

4.1	**Concentration and hydrolysis values**	See Table A7_1_1_1_1-4
4.2	**Hydrolysis rate constant (k_h)**	k (s^{-1}) pH 4: 1.00702 x 10^{-8} pH 7: 1.09222 x 10^{-8} pH 9: 1.31740 x 10^{-8}
4.3	**Dissipation time**	DT_{50} (s) pH 4: -68831451.645 pH 7: -63462367.885 pH 9: -52614804.419
4.4	**Concentration – time data**	The concentration of the test substance at each sampling point expressed as percentage of initial concentrations is given in Table A7_1_1_1_1-4
4.5	**Specification of the transformation products**	Since no degradation has been observed transformation products do not have to be specified

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The hydrolytic stability of p-chloro-m-cresol was tested in accordance with EC Guideline 92/69/EC, C.7 at pH levels of 4, 7, and 9 and 50 °C in

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products (01)

Annex Point IIA7.6.2.1

		buffer solutions. The test duration was 5 days with sampling intervals after 0, 1, 24, 96, and 120 hours.
5.2	**Results and discussion**	p-Chloro-m-cresol was stable under acidic (pH 4), neutral (pH 7) and alkaline (pH 9) conditions at 50°C. Due to HPLC analysis p-chloro-m-cresol accounted for about 100 % in the solutions at termination of the experiments compared to initially applied amounts. Formation of hydrolysis products was not observed in the course of the study. Considering the high hydrolytic stability determined under stringent temperature conditions and at different pH values it is not expected that hydrolytic processes will contribute to the degradation of p-chloro-m-cresol in the environment.
5.2.1	k_h	A value for k_h is given in the report however, since no degradation is observable this is not meaningful
5.2.2	DT_{50}	A value for DT_{50} is given in the report however, since no degradation is observable this is not meaningful
5.2.3	r^2	A value for r is given in the report however, since no degradation is observable this is not meaningful
5.3	**Conclusion**	Validity criteria can be considered as fulfilled.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No duplicate samples have been investigated at each sampling point

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_1_1_1-1: Type and composition of buffer solutions

pH	Type of buffer	Source
4	Citric acid/~~sodium~~ **potassium** hydroxide/sodium chloride	Riedel de Haen Art. No. 33543, ready to use solution
7	Potassium dihydrogen phosphate/ di-sodium hydrogen phosphate	Riedel de Haen Art. No. 33546, ready to use solution
9	Borax/hydrochloric acid	Riedel de Haen Art. No. 33548, ready to use solution

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Not reported
Preparation of test medium	A p-chloro-m-cresol stock solution was prepared by dissolving 1370 mg into a 100 mL volumetric flask, which was filled to the mark with acetonitrile. For each pH value, a 250 mL Erlenmeyer flask was flushed with argon to remove the oxygen than filled with 1 mL of the stock solution, 100 mL buffer solution was added, argon was passed through he solution for 5 min to remove the oxygen. The solutions were agitated in a closed dark shaking heat regulator to avoid any photolytic effect. The used buffer solutions were considered as sterilized at the applied temperatures for the time of the test.
Test concentrations (mg a.i./L)	136 mg/L
Temperature (°C)	50 ± 0.5 °C
Controls	Not reported
Identity and concentration of co-solvent	Acetonitrile
Replicates	None

Table A7_1_1_1_1-3: Description of test system

Glassware	100 mL volumetric flask for the stock solution preparation 250 mL erlenmeyer flasks for the test solution preparation
Other equipment	Agitation of the solutions in a closed dark shaking heat regulator
Method of sterilization	The used buffer solutions were considered as sterilised at the applied temperature for the time of the test.

Table A7_1_1_1_1-4: Hydrolysis of p-chloro-m-cresol at pH 4, 7 and 9, respectively after different incubation times (50°C)

Incubation time (h)	C_t/C_0		
	pH 4	**pH 7**	**pH 9**
0	100.0	100.0	100.0
1	99.7	100.0	99.7
24	100.6	100.6	100.6
96	101.1	100.9	100.9
120	99.9	100.2	100.2
Statistical evaluation			
k (s^{-1})	1.00702×10^{-8}	1.09222×10^{-8}	1.31740×10^{-8}
DT_{50} (s)	-68831451.645	-63462367.885	-52614804.419
r	0.34589	0.54869	0.54874

C_t = concentration at time point t; C_0 = initial concentration

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (01)

Annex Point IIA7.6.2.2

Official use only

		1 REFERENCE
1.1	**Reference**	Wilmes, R. (1988): Tests to determine the photodegradation of 4-chloro-3-methylphenol (Preventol CMK) in water. Determination of the quantum yield of direct photodegradation in water in polychromatic light (ECETOC method). Bayer AG, Sector 5. Agrochemicals Business Group, PF-F/CE-ME, Monheim, Germany, Date: 1988-05-30.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes; ECETOC (1984): Technical report No. 12
2.2	**GLP**	No
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	4-Chlor-3-methylphenol (p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	Product no. [redacted]
3.1.2	Specification	Not relevant
3.1.3	Purity	[redacted]
3.1.4	Radiolabelling	No
3.1.5	UV/VIS absorption spectra and absorbance value	UV/VIS absorption spectra and extinction data are given in the report: the substance has a long wave maximum at 279 nm , whose tail extends to about 300 nm
3.1.6	Further relevant properties	-
3.2	**Reference substances**	Not available from the report
3.3	**Test solution**	See Table A7_1_1_1_2-1
3.4	**Testing procedure**	
3.4.1	Test system	Three tests have been conducted (see Table A7_1_1_1_2-2): 1. Test 1: The direct phototransformation of p-chloro-m-cresol (2.65 mg/L) was determined in a rotating radiation apparatus

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (01)

Annex Point IIA7.6.2.2

		with quartz cuvettes and a Duran 50 filter. The number of photons was determined actinometrically. 2. Test 2 The indirect phototransformation of the test substance was determined by the addition of 10 and 100 mg/L humic acid to the test solution and irradiation with a high pressure mercury vapour lamp. 3. Test 3 A photodegradation test in sunlight with and without addition of 10 mg/L soluble humic acids has been conducted.	
3.4.2	Properties of light source	See Table A7_1_1_1_2-2	
3.4.3	Determination of irradiance	Uranyloxalate actinometer	
3.4.4	Temperature	Not reported	
3.4.5	pH	Not reported	
3.4.6	Duration of the test	Test 1: 8 hours (first replicate), 7.25 hours (second replicate) Test 3: 4 hours Test 3: 32.75 hours	X
3.4.7	Number of replicates	The direct phototransformation test (test 1) was investigated in two experiments	
3.4.8	Sampling	Test 1: 0, 0.66, 1.5, 2.83 and 8 hours (first replicate) 0, 1.66, 2.75, 3.66, 5.25 and 7.25 hours (second replicate) Test 2: 0, 1.33, 2.58 and 4 hours Test 3: 3.25, 7.08, 13.25, 16.75, 23.00 and 32.75 hours	X
3.4.9	Analytical methods	Identity of the test substance: GC, IR-spectrum Assay of p-chloro-m-cresol photolysis in water: HPLC with UV detection Instrument: HP 1090 with workstation Detection: UV, 230 nm (reference wavelength: 550 nm) Column: Merck LiChrosorb RP 18, 5 µm, 250 mm x 4 mm) Flow-rate: 1 mL/min Solvent system: A: 55% water (5% acetonitrile, 0.2% phosphoric acid) B: 45 % acetonitrile Limit of determination: < 0.1 mg/L	
3.4.10	Method of calculation	The environmental half-life was assessed by means of the arithmetic model developed by Zepp & Cline, 1977 (Environ. Sci. Technol. 11, 359). The conditions of the model are: clear sky, pure water close to the surface, 10th longitude, values integrated over the whole day	
3.5	**Transformation products**	No	
3.5.1	Method of analysis for transformation	Not relevant (see Point 3.5)	

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (01)

Annex Point IIA7.6.2.2

products

4 RESULTS

4.1	**Screening test**	The UV spectrum of p-chloro-m-cresol in water has a long wave maximum at 279 nm, whose tails extend to about 300 nm
4.2	**Actinometer data**	Not repoted
4.3	**Controls**	No degradation in dark control samples
4.4	**Photolysis data**	
4.4.1	Concentration values	The course of the test substance concentrations when exposed to light are summarised in Table A7_1_1_1_2-3 (test 1 and 2) and in Table A7_1_1_1_2-4 (test 3)
4.4.2	Mass balance	Not relevant
4.4.3	k^c_p	Test 1, first experiment: 0.2235 h^{-1} Test 1, second experiment: 0.1969 h^{-1} Test 2, 10 m/L humic acid: 0.1945 h^{-1}
4.4.4	Kinetic order	Pseudo first order
4.4.5	k^c_p / k^a_p	K^a_p not reported
4.4.6	Reaction quantum yield (ϕ^c_E)	0.9 (mean of both test 1 experiments)
4.4.7	k_{pE}	Not reported
4.4.8	Half-life ($t_{1/2E}$)	The half-lives for p-chloro-m-cresol amount to 3.1 – 3.56 hours experimentally determined for direct and indirect phototransformation (artificial light, tests 1 and 2) 20.7 – 46.3 hours experimentally determined for indirect phototransformation (natural sunlight, test 3) 31 – 89 days calculated for direct phototransformation in the environment (50°latitude, spring to autumn) For detailed information see Table A7_1_1_1_1-5, Table A7_1_1_1_1-6 and Table A7_1_1_1_1-7
4.5	**Specification of the transformation products**	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test was performed according to ECETOC Technical report No. 12 (1984). Additionally, the indirect phototransformation of the test substance and the phototransformation under natural sunlight was determined.
5.2	**Results and discussion**	p-Chloro-m-cresol is degraded by light with a high quantum yield (0.9). However, it absorbs ultraviolett light to only a very small extent in the sunlight spectrum. Experimentally determined half-lives for direct phototransformation amount to 3.10 and 3.51 hours. Further explanatory

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (01)

Annex Point IIA7.6.2.2

		tests for indirect phototransformation gave a half-life (DT_{50} = 3.56 hours) similar to those of the direct phototransformation. The quantum yield and UV-absorption data were used to estimate the environmental half-life of p-chloro-m-cresol concerning direct photodegradation in water by a simulation model. The results of the modelling indicate that half-lives during the seasons spring to autumn range from one to three months (50° latitude). However, as could be demonstrated by a photodegradation test under natural sunlight conditions, secondary photodegradation induced by soluble humic acids may enhance the dissipation of the compound by sunlight to a high extent. The test gave extrapolated half-lives of the order of 1 to 2 days, as compared with a calculated minimum half-life of 31 days for direct photodegradation.
5.2.1	k^c_p	Test 1, first experiment: 0.2235 h^{-1} Test 1, second experiment: 0.1969 h^{-1} Test 2, 10 m/L humic acid: 0.1945 h^{-1}
5.2.2	K_{pE}	Not reported
5.2.3	ϕ^c_E	0.9 (mean of both test 1 experiments)
5.2.4	$t_{1/2E}$	The half-lives for p-chloro-m-cresol amount to 3.1 – 3.56 hours experimentally determined for direct and indirect phototransformation (artificial light, tests 1 and 2) 20.7 – 46.3 hours experimentally determined for indirect phototransformation (natural sunlight, test 3) 31 – 89 days calculated for direct phototransformation in the environment (50°latitude, spring to autumn)
5.3	**Conclusion**	p-Chloro-m-cresol is degraded by light with a high quantum yield (0.9). As, however, it absorbs ultraviolett light to only a very small extent in the sunlight spectrum, direct photodegradation under environmental conditions is greatly restricted and also depends very much on latitude, time of year and weather conditions. Secondary photodegradation induced by soluble humic acids may enhance the dissipation of the compound by sunlight to a high extend.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	Deficiencies regarding the description of the irradiation apparatus (emission wavelength spectrum and nature of the light source), the actinometer measurement, the laboratory equipment, the conditions of the tests (temperature and pH of the test solution) and the light intensity of natural sunlight.

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Remarks	[redacted]
	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 A7_1_1_1_2-1: Description of the test solution

Criteria	Details
Purity of water	Highly pure water (taken from a Milli-Q-unit, Millipore Co.)
Preparation of test chemical solution	4-Chloro-3-methylphenol was stirred in pure water. After being filtered through a folded filter the concentrated solution was diluted with pure water to 2.65 mg/L (1.86 x 10^{-5} mol/L) In the tests to assess indirect phototransformation in the laboratory, 10 mg/L and 100 mg/L humic acid (potassium salt) were added to the test solution (test 2). In the photodegradation test in natural sunlight one of the test solutions also gained 10 mg/L humic acid (test 3)
Test concentrations (mg a.s./L)	Nominal: 2.65 mg/L (1.86 x 10^{-5} mol/L)
Temperature (°C)	Not reported
Controls	Duplicate samples have been irradiated Dark control samples
Identity and concentration of co-solvent	No co-solvent used, test substance is directly diluted in water

Table A7_1_1_1_2-2: Description of test system

Criteria	Test for direct phototransformation (test 1)	Test for indirect phototransformation (test 2)	Photodegradation in sunlight (test 3)
Test apparatus	Rotating radiation apparatus, quartz cuvettes and Duran 50 filter	Rotating radiation apparatus, quartz cuvettes and Duran 50 filter	Not relevant
Properties of artificial light source:			
Nature of light source	Polychromatic light	High pressure mercury vapour lamp	Not relevant
Emission wavelength spectrum	> 295 nm	> 295 nm	Not relevant
Actinometer	Uranyloxalate actinometer	Not reported	Not relevant
Light intensity	3.19×10^{16} photons/sec and 3 mL	Not reported	Not relevant
Filters	Duran 50 filter to cut off wavelength < 295 nm	Duran 50 filter to cut off wavelength < 295 nm	Not relevant
Properties of natural sunlight:	Not relevant	Not relevant	The test lasted for 4 days: 18/19/21 April 1988: sunny, cloudless 20 April 1988: overcast, rainy

Table A7_1_1_1_2-3: Course of test substance concentration during irradiation (tests 1 and 2)

Direct photolysis (replicate 1)		Direct photolysis (replicate 2)		Indirect photolysis (addition of 10 mg/L humic acid)	
Exposure time (h)	Test substance concentration (mg/L)	Exposure time (h)	Test substance concentration (mg/L)	Exposure time (h)	Test substance concentration (mg/L)
0	2.64	0	2.63	0	2.67
0.66	2.39	1.66	1.91	1.33	2.18
1.5	1.98	2.75	1.5	2.58	1.61
2.83	1.69	3.66	1.24	4	1.25
8	0.45	5.25	1.12	-	-
-	-	7.25	0.58	-	-

Table A7_1_1_1_2-4: Course of test substance concentration during irradiation by natural sunlight (tests 3)

Exposure time (h)	% Degradation	
	Pure water	10 mg/L humic acid
3.25	n.n.	12.3
7.08	n.n.	21.2
13.25	n.n.	25.8
16.75	n.n.	31.0
23.00	n.n.	34.9
32.75	n.n.	41.8

Table A7_1_1_1_2-5: Experimentally determined DT_{50} values for direct (test 1) and indirect phototransformation (test 2)

Parameter	Direct phototrans-formation (test 1), first experiment	Direct phototrans-formation (test 1), second experiment	Indirect phototrans-formation (test 2), addition of 10 mg/L humic acid
DT50 (h)	3.1	3.51	3.56
No. of values	5	6	4
Velocity constant (h^{-1})	0.2235	0.1969	0.1945
r^2	0.9894	0.9896	0.9932

Table A7_1_1_1_2-6: Experimentally determined DT_{50} values for indirect phototransformation and exposure to natural sunlight (test 3)

Test duration (days)	No. of samplings	DT50 (h)	r^2
1	2	20.7	0.9888
2	4	34.0	0.9373
3	5	39.0	0.9346
4	6	46.3	0.9310

Table A7_1_1_1_2-7: Environmental DT_{50} values calculated according to the program developed by Zepp and Cline

Latitude	DT50 values (d) in the seasons			
	Spring	Summer	Autumn	Winter
30	13	9	20	39
40	19	12	36	111
50	31	15	89	503
60	56	21	317	4260

Section A7.1.1.2.1 Ready biodegradability (01, 02, 04)

Annex Point IIA7.6.1.1

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1 REFERENCE

1.1	**Reference**	Müller (1992): Investigations of the ecological behaviour of Preventol CMK Bayer AG, Institut für Umweltanalyse und Bewertungen, Leverkusen, Germany, Report No. A 330 A/91, unpublished, Date: 1992-02-25 Weyers, A. (2007): Preventol CMK – Biodegradation. Re-Evaluation based on Study Report 330 A/91, corresponding raw data and additional information provided by the sponsor. Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany, Report No. A 330 A/91, Date: 2007-03-09; amended: 2007-03-16 Neuhahn, A. (2012): 2. Amendment to GLP-Final Report Study Title: Biodegradation. Re-evaluation based on study report 330 A/91. CURRENTA GmbH & Co. OHG, Analytic, Chempark, building K 46, Leverkusen, Germany.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, Commission Directive 84/449 EEC (Official Journal of the EC No. L 251 of September 10, 1984), Appendix V C.6: Closed Bottle Test. This test method is in all essential parts identical with OECD Guideline 301 D:
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	-
3.2	**Reference substance**	Yes, aniline

Section A7.1.1.2.1 Ready biodegradability (01, 02, 04)

Annex Point IIA7.6.1.1

3.2.1	Initial concentration of reference substance	2 mg/L
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	See Table A7_1_1_2_1-1
3.3.2	Test system	The test system is described in Table A7_1_1_2_1-2.
3.3.3	Test conditions	The test conditions are described in Table A7_1_1_2_1-3
3.3.4	Method of preparation of test solution	The method of the preparation of the test solution is described in Table A7_1_1_2_1-3. Determinations were carried out in duplicate.
3.3.5	Initial TS concentration	4.5 mg/L
3.3.6	Duration of the test	28 days
3.3.7	Analytical parameter	BOD in relation to ThOD
3.3.8	Sampling	The sampling intervals were: 0, 5, 15 and 28 days
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	Not applicable
3.3.11	Controls	Not reported
3.3.12	Statistics	Not relevant

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	The results are presented in tabular form (Table 7_1_1_2_1-4 to Table 7_1_1_2_1-8)
4.1.2	Degradation	The dissolved oxygen content (mg O_2 after x days) of the blank samples is reported in Table 7_1_1_2_1-4. The dissolved oxygen content (mg O_2 after x days) of the blank inoculum samples is reported in Table 7_1_1_2_1-5. The dissolved oxygen content (mg O_2 after x days) of the reference samples is reported in Table 7_1_1_2_1-6. The dissolved oxygen content (mg O_2 after x days) of the test item samples is reported in Table 7_1_1_2_1-7. In Table 7_1_1_2_1-8 the % degradation of CMK have been assessed. CMK degraded by 4.3 % (after 5 days), 78.2% (after 15 days) and 84.5% (after 28 days). All figures are means of two replicates. The standard deviations of the replicates accounted for 6.0%, 6.7%, and 8.3%, respectively. X

Section A7.1.1.2.1 Ready biodegradability (01, 02, 04)

Annex Point IIA7.6.1.1

		A theoretical oxygen demand of 1795 mg O_2/L was calculated.
4.1.3	Other observations	None
4.1.4	Degradation of TS in abiotic control	Not reported
4.1.5	Degradation of reference substance	The dissolved oxygen content (mg O_2 after x days) of the reference samples is reported in Table 7_1_1_2_1-6. In the parallel preparations with the reference substance aniline complete degradation was obtained after 15 days.
4.1.6	Intermediates/ degradation products	Not identified (*cf.* Point 3.3.9)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test on ready biodegradability of p-chloro-m-cresol was conducted according to Commission Directive 84/449 EEC (Official Journal of the EC No. L 251 of September 10, 1984), Appendix V C.6: Closed Bottle Test, which is more or less identical with OECD Guideline 301 D. A solution of p-chloro-m-cresol in a mineral medium was inoculated with effluent of a laboratory scale unit receiving domestic wastewater of a municipal wastewater treatment plant. Incubation lasted for 28 days under aerobic conditions in the dark at 20 – 21 °C. During this period the biodegradation was followed by analysis of dissolved oxygen. Sampling was conducted at appropriate intervals to include the 10-d window.
5.2	**Results and discussion**	p-Chloro-m-cresol degraded by 4.3% (BOD/ThOD) within the first 5 days. Already after 15 days 78.2% biodegradation was reached. The standard deviation of replicate samples was below 9% for sampling intervals of 5, 15, and 28 days. Hence, the test substance can be considered to be readily biodegradable under the conditions of the test.
5.3	**Conclusion**	The criteria for validity of the test are met (*cf.* Table A7_1_1_2_1-9) . p-Chloro-m-cresol can be evaluated to be readily biodegradable under the conditions of the test (Closed Bottle Test)
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	None

X

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*11-06-12*
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 A7_1_1_2_1-1: Inoculum

Criteria	Details
Nature	Activated sludge
Species	Mixed population of aquatic microorganisms
Source/Sampling site	Effluent of a laboratory scale unit receiving exclusively domestic sewage from the Wupper area water authority. The domestic sewage of the Wupper area authority was pumped into the laboratory scale unit in the wastewater treatment plant Leverkusen-Bürrig. The scale unit was run with domestic wastewater as the influent. The effluent of this laboratory scale unit was taken and transported immediately to the testing facility. An adaption of the micro-organisms to industrial chemicals did not take place.
Sampling date	1991-12-13
Laboratory culture	No
Method of cultivation	Not relevant
Preparation of inoculum for exposure	Course particles were separated by filtration. The first 200 mL were decanted and the remaining filtrate was aerated for 3 to 5 days until use as inoculum.
Pretreatment	None
Initial cell concentration	Effluent concentration in reaction mixture: 5 mL/L

Table A7_1_1_2_1-2: Test system

Criteria	Details
Culturing apparatus	None
Number of culture flasks/concentration	32 BOD bottles, 250-300 mL, with glass stoppers: The following types of flasks were used: Test suspension: containing inoculated mineral medium and test item solution Procedure control: containing inoculated mineral medium and the reference substance solution Inoculum blank: containing only inoculated mineral medium Blank: containing a measured volume of deionised, oxygen saturated water with mineral medium
Aeration device	During exposure, the suspensions were incubated with permanent aeration. The aeration device is not further specified.
Measuring equipment	Analysis of dissolved oxygen. The measuring equipment is not further specified.
Test performed in closed vessels due to significant volatility of TS	The test was performed as closed bottle test.

Table A7_1_1_2_1-3: Test conditions

Criteria	Details
Composition of medium	The mineral medium was prepared from concentrated stock solutions in demineralised water 1. Mineral salt solution: KH_2PO_4 8.50 g K_2HPO_4 21.75 g Na_2HPO_4 x 2 H_2O 33.30 g NH_4Cl 1.70 g Dissolved in deionised water and made up to 1 L. The pH is adjusted to 7.4. Magnesium sulphate solution: $MgSO_4$ x 7 H_2O 22.50 g Dissolved in water and made up to 1 L. Calcium chloride dihydrate: $CaCl_2$ x 2 H_2O 36.40 g Dissolved in deionised water and made up to 1 L. Iron (III) chloride solution: $FeCl_3$ x 6 H_2O 0.25 g Dissolved in deionised water and made up to 1 L To prepare the mineral medium 25 mL of the magnesium sulphate-, calcium chloride- and the iron (III) chloride solution were transferred to 20 L deionised water. To prepare the inoculated mineral medium 125 mL inoculum were added to mineral medium and filled up to 25 L. The pH is adjusted to 7.2.
Additional substrate	None
Test temperature	20 – 21°C
pH	The inoculated mineral medium was adjusted to pH 7.2.
Aeration of dilution water	During exposure, the suspensions were incubated with permanent aeration. An aeration of dilution water is not reported.
Suspended solids concentration	Not reported
Concentration of inoculum	5 mL/L
Other relevant criteria	None

Table A7_1_1_2_1-4: Dissolved oxygen content (mg O_2 after x days) of blank samples

Replicate	Days of incubation			
	0 days	5 days	15 days	28 days
1	9.06	8.53	8.36	8.41
2	9.06	8.56	8.70	8.34
Mean	9.06	8.545	8.53	8.375

Table A7_1_1_2_1-5: Dissolved oxygen content (mg O_2 after x days) of blank inoculum

Replicate	Days of incubation			
	0 days	5 days	15 days	28 days
1	9.08	8.26	8.37	8.06
2	9.01	8.25	8.17	8.12
Mean	9.045	8.255	8.27	8.09

Table A7_1_1_2_1-6: Dissolved oxygen content (mg O_2 after x days) of reference samples

Replicate	Days of incubation			
	0 days	5 days	15 days	28 days
1	9.07	4.63	2.88	2.10
2	9.08	4.69	2.83	2.44
Mean	9.075	4.66	2.855	2.27

Table A7_1_1_2_1-7: Dissolved oxygen content (mg O_2 after x days) of CMK samples

Replicate	Days of incubation			
	0 days	5 days	15 days	28 days
1	9.00	7.58	1.58	1.76
2	9.08	8.27	2.35	0.80

Table A7_1_1_2_1-8: Biodegradation (%) of p-chloro-m-cresol (4.5 mg/L test substance)

Parameter	Days of incubation		
	5 days	15 days	28 days
$(m_{(0)} - t1_{(x)}) - (mb_{(0)} - mb_{(x)})$	0.69	6.71	6.35
$(m_{(0)} – t2_{(x)}) - (mb_{(0)} - mb_{(x)})$	0.00	5.94	7.31
% degradation replicate 1	8.5	83.0	78.6
% degradation replicate 2	0.0	73.5	90.4
% degradation mean	4.3	78.2	84.5
Standard deviation (%)	6.01	6.72	8.34

Table A7_1_1_2_1-9: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	Yes	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	Yes	
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Yes	
Percentage of removal of reference substance reaches pass level by day 14	Yes	

Section A7.1.1.2.1 Ready biodegradability (03)

Annex Point IIA7.6.1.1

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		1 REFERENCE
1.1	**Reference**	Hanstveit, A.O. & Pullens, M.A.H.L. (1993): The biodegradability of the product Preventol CMK in a closed bottle test according to a draft OECD guideline: ready biodegradability; the influence of inoculum activity TNO Institute of Environmental Sciences, Delft, The Netherlands Report No. R 92/198, unpublished, Date: 1993-01-15 amended: 2007-03-30
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, Draft OECD Guideline for testing of chemicals,ready biodegradability. Doc. No. CDUP/89.104/13.6
2.2	**GLP**	Yes (main test), a limited additional test was conducted under non-GLP conditions
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Chemical Oxygen Demand (COD) = 1.756 mg O_2/mg
3.2	**Reference substance**	Yes, sodium acetate
3.2.1	Initial concentration of reference substance	3.96 mg/L
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_1_2_1-1. In order to prove the results of the main test a limited additional test under non-GLP conditions was conducted using activated sludge from a different source.
3.3.2	Test system	The test systems for the main and the additional tests are described in Table A7_1_1_2_1-2.

Section A7.1.1.2.1 Ready biodegradability (03)

Annex Point IIA7.6.1.1

3.3.3	Test conditions	The test conditions are described in Table A7_1_1_2_1-3. The additional test was conducted under the same conditions as the main test. Only the source of the inocula (see Point 3.3.1) and the inocula concentrations (see also Point 3.3.2) were different.
3.3.4	Method of preparation of test solution	A stock solution was prepared by adding 54.6 mg of the test substance to 3.2 L of inoculated mineral medium. A test concentration of 1.71 mg/L was prepared by adding 600 mL of the stock solution to 5.4 L of inoculated mineral medium.
3.3.5	Initial TS concentration	1.71 mg/L
3.3.6	Duration of the test	Main test: 56 days, except the toxicity control which ended after 28 days Additional test: 21 days
3.3.7	Analytical parameter	BOD
3.3.8	Sampling	Main test: The sampling intervals were: 0, 7, 14, 21, 28, 42 and 56 days Toxicity control: 0, 7, 14, 21 and 28 days Additional test: 0, 7, 21 days
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	Not applicable
3.3.11	Controls	Main test: Toxicity control: 1.71 mg/L test substance, 3.96 mg/L sodium acetate, either 2.5 or 7.6 mL/L inoculum, mineral medium Background oxygen consumption: inoculated mineral medium without test substance Biological activity control: 3.96 mg/L sodium acetate, either 2.5 or 7.6 mL/L inoculum and mineral medium Additional test: Background oxygen consumption: 5 mL/L inoculum, no test substance, mineral medium
3.3.12	Statistics	Not relevant
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	The results are presented in tabular form (Table A7_1_1_2_1-4)

Section A7.1.1.2.1 Ready biodegradability (03)

Annex Point IIA7.6.1.1

4.1.2	Degradation	The biodegradation during the main test is shown in Table A7_1_1_2_1-4. A chemical oxygen demand (COD) of 1.756 mg O_2/mg was calculated. Table A7_1_1_2_1-5 is a summary of the oxygen concentration and the BOD during the confirmatory additional test.
4.1.3	Other observations	The calculated oxygen consumption of acetate in the presence of the test substance was similar or lower than that of acetate alone during 28 days of incubation in the main test (*cf.* Table A7_1_1_2_1-6).
4.1.4	Degradation of TS in abiotic control	Not reported. It was assessed that the test substance was stable to oxidation in the mild conditions of the Closed Bottle Test. The lack of oxidation of the test substance was confirmed in the additional test with inoculated medium in which no significant oxygen uptake due to degradation of the test substance was recorded within 21 days.
4.1.5	Degradation of reference substance	The results of the determination of the oxygen concentrations in the control tests (only 3.96 mg/L sodium acetate) and the toxicity tests (3.96 mg/L sodium acetate plus 1.71 mg/L test substance) are presented in Table A7_1_1_2_1-6.
4.1.6	Intermediates/ degradation products	Not identified (*cf.* Point 3.3.9)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The biodegradability of p-chloro-m-cresol was determined in a 56 days test by the “Closed Bottle Test” method described in the Draft OECD Guideline for testing of chemicals using oxygen consumption as the test criterion. Objective of the study was the determination of the ready biodegradability of the test substance at two different inoculum activities. An additional test (under non-GLP conditions) was carried out in order to confirm the results of the first 21 days of the test.

Section A7.1.1.2.1 Ready biodegradability (03)

Annex Point IIA7.6.1.1

5.2	**Results and discussion**	The oxygen consumption of the inoculated mineral medium (without test and reference substance) after 28 days was 1.00 and 1.91 mg O_2/L for the low and high inoculum concentration, respectively. The former value is lower than the maximum value of 1.5 mg O_2/L prescribed in the Guideline. The difference between the oxygen consumption of the two inocula was mostly a factor of 2. However, at the end of the test (after 56 days) the difference between the two inocula was only a factor of 1.2, although the remaining oxygen in the bottles was sufficient for a greater difference. Sodium acetate was degraded completely within 7 days. The measured oxygen consumption of acetate after 7 days was 0.53 mg O_2/mg which compares well with the ThOD of 0.68 mg O_2/mg and corresponds to 80% degradation. The calculated oxygen consumption of sodium acetate in the presence of p-chloro-m-cresol was similar or somewhat lower than that of acetate during 28 days, indicating that acetate degradation was slightly inhibited by the tested concentration of p-chloro-m-cresol. In the biodegradation test the oxygen consumption which could be attributed to p-chloro-m-cresol started between day 28 and 42. After 56 days a biodegradability of 32 % was found for the low inoculum activity (2.5 mL/L) and 52% with the high inoculum activity (7.6 mg/L). Due to the lack of degradation during the first three weeks, a limited additional test was carried out under the same conditions as the main test. The additional test corroborated the lack of degradation during the first part of the study.	X
5.3	**Conclusion**	The tested concentration of p-chloro-m-cresol might have inhibited the endogenous activity of the inoculum during the first three weeks. However, according to the validity criteria for tests conducted according to OECD Guideline 301, test substances are inhibitory if in the toxicity test, containing both the test and the reference compounds less than 25% degradation (based on ThOD) occurs within 14 days. Since this is not the case for p-chloro-m-cresol, the compound cannot be denoted as inhibitory at the concentrations tested. After the three weeks adaption period, a significant degradation of the test compound could be noticed. The length of the adaption period appears to depend on the inoculum activity. The extent of biodegradability of the test compound was also dependent on the inoculum activity, i.e., p-chloro-m-cresol is to a higher degree biodegradable at a higher inoculum activity compared to a lower inoculum activity. Under the conditions of this Closed Bottle Test, p-chloro-m-cresol is not readily biodegradable.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_1_2_1-1: Inoculum

Criteria	Details
Nature	Activated sludge
Species	Mixed population of aquatic microorganisms
Source/Sampling site	Main test: Oxidation ditch situated on the premises of TNO, Delft, The Netherlands. The oxidation ditch is used to treat domestic sewage. Additional test: Oxidation ditch in Berkel-Rodenrijs county
Preparation of inoculum for exposure	Main test: The sludge was allowed to settle for 5-7 minutes. Then 125 and 400 mL respectively of the supernatant was used to inoculate 50 L of mineral medium
Pretreatment	None
Initial cell concentration	Main test: 3.90 g of solid substance per L (original sludge)

Table A7_1_1_2_1-2: Test system

Criteria	Details
Culturing apparatus	Not specified
Number of culture flasks/concentration	Main test: 19 bottles: 1.71 mg/L test substance, 2.5 mL/L inoculum, mineral medium 19 bottles: 1.71 mg/L test substance, 7.6 mL/L inoculum, mineral medium 13 bottles: toxicity control: 1.71 mg/L test substance, 3.96 mg/L sodium acetate, either 2.5 or 7.6 mL/L inoculum, mineral medium 19 bottles: inoculated mineral medium without test substance for measuring background oxygen consumption A not specified number of bottles was prepared containing 3.96 mg/L sodium acetate, either 2.5 or 7.6 mL/L inoculum and mineral medium Additional test: A not specified number of bottles was prepared containing 1.71 mg/l test substance, 5 mL/L inoculum and mineral medium A not specified number of bottles was prepared containing 5 mL/L inoculum and mineral medium for measuring background oxygen consumption
Aeration device	Not relevant
Measuring equipment	Oxygen electrode to measure the oxygen concentration
Test performed in closed vessels due to significant volatility of TS	Not relevant

Table A7_1_1_2_1-3: Test conditions

Criteria	Details
Composition of medium	The mineral medium was prepared from concentrated stock solutions in demineralised water Stock solution 1: KH_2PO_4 8.50 g K_2HPO_2 21.75 g Na_2HPO_4 x 7 H_2O 50.30 g NH_4Cl 0.50 g Dissolved in water and made up to 1 L Stock solution 2: $CaCl_2$: 27.50 g or $CaCl_2$ x 2 H_2O 36.40 Dissolved in water and made up to 1 L Stock solution 3: $MgSO_4$ x 7 H_2O 22.50 Dissolved in water and made up to 1 L Stock solution 4: $FeCl_3$ x 6 H_2O 0.20 Dissolved in water and made up to 1 L 1 mL of each stock solution (1 to 4) is mixed and made up to 1 L with Milli Q water
Additional substrate	None
Test temperature	19.1 – 20.4°C in the dark
pH	6.6 – 7.0
Aeration of dilution water	Yes, the dilution water was aerated vigorously before use
Suspended solids concentration	Main test: 3.90 g solid substance/L in the original sludge
Concentration of inoculum	Main test: 2.5 and 7.6 mL/L Additional test: 5.0 mL/L
Other relevant criteria	None

Table A7_1_1_2_1-3bis: O_2 concentrations and corresponding O_2 uptakes for p-chloro-m-cresol (1.71 mg/L test substance) at two inoculum activities (main test)

Time (days)	Inoculum activity 2.5 mL/L				Inoculum activity 7.6 mL/L			
	Control		1.71 mg/L CMK		Control		1.71 mg/L CMK	
	O_2 conc (mg/L)	O_2 uptake (mg/L)	O_2 conc (mg/L)	O_2 uptake (mg/L)	O_2 conc (mg/L)	O_2 uptake (mg/L)	O_2 conc (mg/L)	O_2 uptake (mg/L)
0	9.25	-	9.20	-	9.28	-	9.16	-
7	8.51	0.69	8.67	0.53	7.99	1.21	8.65	0.55
14	8.47	0.73	8.88	0.32	7.73	1.47	8.45	0.75
21	8.00	1.20	8.31	0.89	7.48	1.72	7.99	1.21
28	8.20	1.00	8.08	1.12	7.29	1.91	7.25	1.95
42	6.95	2.25	6.84	2.36	6.42	2.78	6.07	3.13
56	6.61	2.59	5.63	3.57	6.07	3.13	4.50	4.70

Table A7_1_1_2_1-4: Biodegradability of p-chloro-m-cresol (1.71 mg/L test substance) at two inoculum activities (main test)

Time (days)	Inoculum activity 2.5 mL/L		Inoculum activity 7.6 mL/L	
	BOD (mg O_2/mg)	% biodegradation*	BOD (mg O_2/mg)	% biodegradation*
7	< 0	-	< 0	-
14	< 0	-	< 0	-
21	< 0	-	< 0	-
28	0.07	4	0.02	1
42	0.06	3	0.20	11
56	0.57	32	0.92	52

COD of p-chloro-m-cresol = 1.756 mg O_2/mg
*% biodegradation = (BOD/COD) x 100

Table A7_1_1_2_1-5: Oxygen concentrations and BOD in the limited additional test (5 mL/L inoculum)

Time	Control (no p-chloro-m-cresol)		1.71 mg/L p-chloro-m-cresol	
	mg O_2/L	BOD (mg/L)	mg O_2/L	BOD (mg/L)
0	8.57	-	8.54	-
7	8.42[a)]	0.15	8.47[a)]	0.07
21	8.03[b)]	0.54	7.97[b)]	0.57

[a)] two bottles; [b)] three bottles

Table A7_1_1_2_1-6: Oxygen concentration and BOD values in the control tests with sodium acetate (3.96 mg/L*) at two inoculum activities and test substance concentrations (0 and 1.71 mg/L)

Preventol CMK (mg/L)	Inoculum concentration mL/L	BOD (mg/mg) after 7 days	BOD (mg/mg) after 14 days	BOD (mg/mg) after 21 days	BOD (mg/mg) after 28 days
0	2.5	0.53	0.55	0.60	0.64
1.71	2.5	0.57	0.54	0.58	0.56
0	7.6	0.50	0.56	0.57	0.71
1.71	7.6	0.61	0.65	0.60	0.61

*ThOD of sodium acetate = 0.68 mg O_2/mg

Table A7_1_1_2_1-7: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled
Pass levels	
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	No
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	No
Criteria for validity	
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Yes
Percentage of removal of reference substance reaches pass level by day 14	Yes

Section 7.1.1.2.1	**Ready biodegradability (06)**
Annex Point IIA7.6.1.1	
Reference	Cernik 1999, A study of biodegradability of 4-chloro-4-methylphenol by aerobic biological treatment. Thesis submitted to the School of Environmental Science and Management to Complete Requirements for a Masters of Science Degree. Duquesne University
Study summary	Cernick (1999) investigated the biodegradability of CMK by means of respirometry. A Micro Oxymax instrument was used, capable of detecting the test substance induced oxygen consumption and carbon dioxide production by a mixed population of microorganisms. The percent O_2 and CO_2 gas levels of the test chamber environment were measured periodically over 450 hours. The acclimated biomass for the bench scale aerobic test reactor was obtained at Bayer Corporation's New Martinsville facility, biological system aerator which is representative of a typical industrial waste treatment facility. A chamber, containing seed without test substance was established as a control. For a test solution of 74.3 mg/L CMK a ThOD of 133 mg/L was calculated. The experiment came to the result of 10 % degradation (13.3 mg/L oxygen consumption) being reached after 5.96 days. The 70% degradation level of 93.1 mg/L oxygen consumption was achieved by 9.54 days and the pass level of 60 % degradation occurred in 3.58 days which is well within the 10-day window. The test does not follow adequate guidelines and is overall considered as not valid for ready biodegradability as the biomass used was obtained from an industrial waste treatment system.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/11/11
Evaluation of applicant's justification	[redacted]
Conclusion	
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 A7.1.1.2.2 Inherent biodegradability (01)

Annex Point IIA7.6.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Thompson, R.S. (1993): Parachlorometacresol: Further study of inherent biodegradability. Brixham Environmental Laboratory, Zeneca limited, Brixham Devon, UK, Report No. BL4783/B, unpublished, Date: 1993-06-29	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 302 A (1981); Inherent biodegradability: Modified SCAS test OECD Guideline 301 B (1981); Ready biodegradability: Closed bottle test	X
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-radiolabelled test substance parachlorometacresol (p-chloro-m-cresol, Preventol CMK)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Non-radiolabelled test substance	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Theoretical Oxygen Demand (ThOD) = 1.85 g O_2/g	X
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not relevant	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_1_2_2-1.	
3.3.2	Test system	The test system is described in Table A7_1_1_2_2-2.	
3.3.3	Test conditions	The test conditions are described in Table A7_1_1_2_2-3.	
3.3.4	Method of	Solutions of the test substance were prepared in deionised water. The	

Section A7.1.1.2.2 Inherent biodegradability (01)

Annex Point IIA7.6.1.2

	preparation of test solution	stock solution contained the test substance at a concentration of 25 mg/L. SCAS unit: each day, 500 mL of the supernatant were drawn off. Afterwards the units were made up to 1 litre volume with fresh sewage after the addition of 100 mL of the sock solution to give a concentration of 5 mg/L. Closed bottle test: Solutions of the test substance at a concentration of 5 mg/L were incubated
3.3.5	Initial TS concentration	SCAS test: 0.5 mg/L for the first 7 days and 5 mg/L thereafter Closed bottle test: 5 mg/L
3.3.6	Duration of the test	SCAS test: 58 days Closed bottle test: 28 days
3.3.7	Analytical parameter	Semi-continuous activated sludge (SCAS) system: The test substance concentration was too low to allow reliable determinations of biodegradability by carbon removal. Therefore, at approximately weekly intervals the effluent from the SCAS unit was analysed for p-chloro-m-cresol. Closed bottle tests: biochemical oxygen demand (BOD)
3.3.8	Sampling	SCAS unit: Sampling for analysis of p-chloro-m-cresol after 7, 9, 14, 21, 27, 35, 43 and 58 days. The test effluent was sampled in triplicate on each occasion. Two 28-day closed bottle tests were started: one used the microbial inoculum from the SCAS unit after 16 days of SCAS acclimatisation, the other one used the microbial inoculum of the 38 days adapted micro-organisms. Each closed bottle test was sampled after 5, 10, 15, 20 and 28 days
3.3.9	Analytical methods	Effluents of the SCAS unit containing the test substance were sampled in triplicate at each occasion and analysed for p-chloro-m-cresol. The test substance stock solution used to prepare the daily feed was also analysed on 6 occasions. The SCAS mixed liquors (approximately 10 minutes after dosing) were sampled on days 9 and 14, and the supernatant analysed after centrifugation. The solutions of the closed bottle tests were analysed at five occasions. The samples were analysed by HPLC using an UV-detector. The samples were quantified against known standards of the test substance. The analytical method and the measuring equipment are described in Table A7_1_1_2_2-2.
3.3.10	Intermediates/ degradation products	Not identified
3.3.11	Nitrate/nitrite measurement	Not applicable

Section A7.1.1.2.2 Inherent biodegradability (01)

Annex Point IIA7.6.1.2

3.3.12	Controls	SCAS unit: One SCAS unit (without test substance) operated in parallel to serve as a control. Furthermore, the non-purgeable dissolved organic carbon content (NPOC) of the settled effluent was determined at intervals in order to ensure that the sludge remained active. Closed bottle test: Toxicity control: 5.0 mg/L test substance, 3.2 mg/L glucose and 3.2 mg/L glutamic acid, 1 mL/L inoculum and mineral medium Background oxygen consumption: 1 mL/L inoculum, no test substance, mineral medium
3.3.13	Statistics	Not relevant

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	The results are presented in tabular form (Table A7_1_1_2_2-4 to Table A7_1_1_2_2-6)
4.1.2	Degradation	The degradation of p-chloro-m-cresol during the SCAS acclimatisation period is shown in Table A7_1_1_2_2-4. Table A7_1_1_2_2-5 is a summary of the oxygen concentration and the BOD during the two closed bottle tests initiated after 16 and 35 days of SCAS acclimatisation.
4.1.3	Other observations	The oxygen concentration and the BOD values in the control tests with glucose/glutamic acid and glucose/glutamic acid/p-chloro-m-cresol are reported in Table A7_1_1_2_2-6).
4.1.4	Degradation of TS in abiotic control	Not reported
4.1.5	Degradation of reference substance	Not relevant, see Point 3.2
4.1.6	Intermediates/ degradation products	Not identified (*cf.* Point 3.3.10)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The inherent biodegradability of p-chloro-m-cresol was determined according to OECD guidelines 302 A and 301 B. A semi-continuous activated sludge (SCAS) system was dosed daily with the test substance at a concentration of 0.5 mg/L for the first 7 days and 5 mg/L until day 58 in order to allow adaption of the microorganisms. After 16 and 35 days microbial inoculum of the SCAS unit was used for a 28-days closed bottle test and the BOD was measured following a single application of the test substance at a concentration of 5 mg/L.

Section A7.1.1.2.2 Inherent biodegradability (01)

Annex Point IIA7.6.1.2

5.2	**Results and discussion**	The non-purgeable dissolved organic carbon content of the settled effluent in the SCAS units was < 30 mg/L, indicating a normal sludge activity throughout the test period. The analysis of the stock solutions used to dose the SCAS system was in good agreement with the nominal values. Within 9 days of adaption in the SCAS unit, approximately 95% removal of the test substance was occurring. The measured concentration of p-chloro-m-cresol in the aqueous phase of the SCAS mixed liquors on day 9 was 70% of the nominal ones, suggesting some degree of sorption to sludge solids. After 14 days the mixed liquor concentration had increased, with a corresponding increase in the effluent concentration, suggesting that the influence of sorption was declining. Effluent concentrations remained relatively stable for the following 2 weeks. After 35 days of SCAS acclimatisation, the effluent concentration was below the detection limit of the analytical method and this was maintained up to day 58. During the first closed bottle test initiated after 16 days of SCAS acclimatisation no significant increase in oxygen consumption compared to the control could be observed. Chemical analysis carried out at three occasions during the test showed no significant removal of the parent compound. During the second closed bottle test initiated after 35 days of SCAS acclimatisation a significant biodegradation was observed after 5 days and 78% biodegradation (BOD in % of ThOD) was achieved after 28 days. Chemical analyses carried out on days 5 and 20 showed > 98% removal of the test substance. The results of the parallel experiments to determine inhibition of glucose/glutamic acid biodegradation by p-chloro-m-cresol showed that the test substance was not inhibitory at 5 mg/L.
5.3	**Conclusion**	It can be concluded that p-chloro-m-cresol is inherently biodegradable. Although some degree of sorption to the sludge solids may have occurred initially, the removal observed in the early stages from the SCAS unit was considerd to be predominantly due to biodegradation. The lack of biodegradation in the first closed bottle test (initiated after 16 days of SCAS acclimatisation) suggests that, at this stage, the microorganisms capable of degrading the test substance in the SCAS were present in insufficient numbers (or were inappropriate species) to do so under the more stringent conditions of the closed bottle test. However, with further acclimatisation of the SCAS population, the inoculum for the second closed bottle test (initiated after 35 days of SCAS acclimatisation) was able to biodegrade p-chloro-m-cresol rapidly and to a high level.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	The degradation of the test substance in an abiotic control is not reported. The measuring equipment of the closed bottle test and the pH during the test are not mentioned.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_1_2_2-1: Inoculum

Criteria	Details
Nature	Activated sludge and sewage
Species	Mixed population of aquatic microorganisms
Source/Sampling site	The activated sludge and sewage were obtained from Buckland Sewage Treatment Works, Newton Abbot, Devon, which receives sewage of primarily domestic origin
Preparation of inoculum for exposure	A suspension of activated sludge in tapwater was added to each SCAS unit to provide 1000 mg mixed liquor suspended solids and made up to 500 mL with tapwater. Settled sewage was added to a total volume of 1 L and aeration was started.
Pretreatment	A SCAS system was used to acclimatise the microorganisms to the test substance. The SCAS unit was dosed with the test substance at a concentration of 0.5 mg/L for the first 7 days and 5 mg/L thereafter.
Initial cell concentration	See above

Table A7_1_1_2_2-2: Test system

Criteria	Details
Semi-continuous activated sludge (SCAS) system	
Test apparatus	Cylindrical glass vessel of 1 litre working volume. A side drain tap at, approximately, the 350 mL level was used for supernatant removal. One test unit received the test substance, a further unit operating in parallel without test substance served as a control
Aeration	Aeration for mixing and oxygenation was provided via a sintered glass disk in the base of the vessel
Number of culture flasks/concentration	Two SCAS units: One unit received the test substance, a further unit was set up and operated in parallel (without test substance) to serve as a control. The test substance concentration in the SCAS unit was 0.5 mg/L for the first 7 days and 5 mg/L thereafter.
Measuring equipment	Analysis of the p-chloro-m-cresol concentration by HPLC using an ultra-violet detector. HPLC equipment and conditions: Column: 100 mm x 4.6 mm id stainless steel Column packing: Spherisorb S5 ODS 2 Eluent: 60:40 methanol:deionised water Eluent flow rate: 2.0 mL/min Wavelength: 225 nm Injection volume: 10 µL
Closed Bottle test	
Laboratory equipment	Standard glass reagent bottles, with ground glass stoppers, of approximately 280 mL capacity
Aeration	Not reported
Number of culture flasks/concentration	15 bottles for measuring BOD 15 control bottles (without test substance) 6 bottles for measuring the p-chloro-m-cresol concentration The test substance concentration was 5 mg/L
Measuring equipment	Not reported

Table A7_1_1_2_2-3: Test conditions

Criteria	Details
Semi-continuous activated sludge (SCAS) system	
Composition of the SCAS	A suspension of activated sluge in tapwater was added to each unit to provide 1000 mg mixed liquor suspended solids and made up to 500 mL with tapwater. Settled sewage was added to a total volume of 1 litre and aeration initiated.
SCAS unit cycle	Each day the aeration was stopped to allow sludge solids to settle to the bottom of the vessel, and 500 mL ot the supernatant (effluent) was drawn off. The SCAS units were made up to 1 litre volume with fresh sewage after the addition of 100 mL of a stock solution of the test substance (25 mg/L) to give a concentration of 5 mg/L in the replaced volume of the test unit. Due to a calculation error, the test unit received 0.5 mg/L in the replaced volume for the first 7 days of the study.
pH of the aerated liquors	> 6.2
Temperature	20 ± 2°C

Table A7_1_1_2_2-3: Test conditions (cont.)

Criteria	Details
Closed Bottle test	
Composition of nutrient solution	Stock solution a): $FeCl_3$ x 6 H_2O 0.2 g Na_2EDTA 0.4 g Deionised water to 1000 mL Stock solution b): $CaCl_2$ x 6 H_2O 54.3 g Deionised water to 1000 mL Stock solution c): $MgSO_4$ x 7 H_2O 22.5 g Deionised water to 1000 mL Stock solution d): KH_2PO_4 8.5 g K_2HPO_4 21.75 g Na_2HPO_4 x 2 H_2O 33.4 g NH_4Cl 0.5 g Deionised water to 1000 mL
Composition of the inoculum	The inoculum used was the supernatant of the settled effluent from the SCAS test unit (acclimatised seed)
BOD dilution water	The BOD dilution water was prepared to contain 1 mL of each stock nutrient solution a) to d) and 1 mL of inoculum per litre of deionised water (Milli-Q grade)
Additional substrate	A further set of bottles was prepared, containing glucose (3.2 mg/L) and glutamic acid (3.2 mg/L) from a stock solution (150 mg/L of each) with a nominal 5-day BOD of 200 mg/L. The dissolved oxygen decrease in these bottles was determined after 5 days.
Test temperature	20°C in the dark
pH	Not reported
Suspended solids concentration	Not reported
Concentration of inoculum	1 mL/L
Other relevant criteria	None

Table A7_1_1_2_2-4: Concentrations of p-chloro-m-cresol in the SCAS system

Sampling day	Concentration of p-chloro-m-cresol (mg/L)				Removal (%)***
	Nominal SCAS concentration*	Measured SCAS concentration	SCAS effluent concentration	SCAS stock solution	
7	0.25	n.m.	< 0.046 < 0.046 < 0.046	25	> 82
8	2.5**	n.m.	n.m.	n.m.	n.m.
9	2.6	1.8 1.8 1.8	0.12 0.13 0.12	n.m.	95
14	2.6	2.0 2.1 2.0	0.19 0.19 0.19	n.m.	93
21	2.6	n.m.	0.22 0.22 0.22	25	92
27	2.6	n.m.	0.12 0.13 0.13	25	95
35	2.5	n.m.	< 0.042 < 0.042 < 0.042	24	> 98
43	2.5	n.m.	< 0.078 < 0.078 < 0.078	24	> 97
58	2.5	n.m.	< 0.085 < 0.085 < 0.085	26	> 97

* Nominal SCAS liquor concentration: mean of dosed concentration (5 mg/L) and effluent concentration
** Nominal concentration increased to 2.5 mg/L (50 % replaced at 5 mg/L)
*** Percent removal = ((1 – effluent concentration)/nominal mixed liquors concentration) x 100
n.m. = not measured

Table A7_1_1_2_2-5: Results of the two closed bottle tests (BOD) initiated after 16 and 35 days of SCAS acclimatisation

Days	Dissoved oxygen (mg/L)		Oxygen demand			PCMC conc. (mg/L)
	Control*	PCMC	mg O_2/L	% ThOD**	Mean % ThOD	
First closed bottle test after 16 days of SCAS acclimatisation						
5	8.8	8.8 8.8 8.8	0 0 0	0 0 0	0	n.m.
5	n.m.	n.m.	n.m.	n.m.	n.m.	4.9 4.8
10	8.9	9.0 9.0 9.0	0 0 0	0 0 0	0	n.m.
11	n.m.	n.m.	n.m.	n.m.	n.m.	4.9
15	8.9	8.8 8.8 8.8	0.1 0.1 0.1	1 1 1	1	n.m.
20	9.0	9.0 9.0 9.0	0 0 0	0 0 0	0	n.m.
27	n.m.	n.m.	n.m.	n.m.	n.m.	4.7 4.7 4.9
28	8.6	8.6 8.6 8.6	0 0 0	0 0 0	0	n.m.
Second closed bottle test after 35 days of SCAS acclimatisation						
5	9.1	4.4 4.8 5.6	4.7 4.3 3.5	51 46 38	45	n.m.
5	n.m.	n.m.	n.m.	n.m.	n.m.	< 0.079
10	8.7	2.4 2.3	6.3 6.4	68 69	69	n.m.
15	8.6	2.1 1.8	6.5 6.8	70 74	72	n.m.
20	8.6	2.2 1.8 1.8	6.4 6.8 6.8	69 74 74	72	< 0.083 < 0.083 < 0.083
28	8.7	1.6 1.4 1.5	7.1 7.3 7.2	77 79 78	78	n.m.

PCMC = p-chloro-m-cresol, ThOD = Theoretical Oxygen Demand, n.m. = not measured
* Control value is the mean of three bottles
**ThOD = 1.85 g O_2/g = 9.25 mg O_2 at PCMC concentration of 5 mg/L

Table A7_1_1_2_2-6: Oxygen concentration and BOD values in the control tests with glucose/glutamic acid and glucose/glutamic acid/PCMC

Treatment	Dissolved oxygen (mg/L) at day 5	Oxygen demand (mg/L)	BOD_5 stock solution (mg/L)*		% inhibition
			Replicates	Mean	
Control (mean)	8.8	n.r.	n.r.	n.r.	n.r.
Control (glucose/glutamic acid)	4.4 4.4 4.4	4.4 4.4 4.4	205 205 205	205	n.r.
PCMC (5 mg/L), glucose/glutamic acid	4.3 4.4 4.7	4.5 4.4 4.1	210 205 191	202	1

n.r. = not relevant, PCMC = p-chloro-m-cresol, BOD = Biological Oxygen Demand
*** Stock solution diluted 6 mL in 280 mL (x 46.67)**

Section 7.1.1.2.3 **Annex Point IIIA 12.2**	**Biodegradation in seawater**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification […].**	
Detailed justification:	[redacted]	
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	March 2008	
Evaluation of applicant's justification	[redacted]	
Conclusion		
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 7.1.2.1.1 **Annex Point IIIA 12.2**	**Biological sewage treatment** **Aerobic biodegradation (10)**
Reference	Morris 2002, Bench scale biological treatment of preventol CMK for General Motor's Lansing Plant #5.
Study summary	The biodegradability of CMK in the wastewater of a metal working fluid plant was investigated by employing a bench scale bioreactor set-up to simulate the plant's existing treatment scheme. The 10-L bioreactors were inoculated with the same biological media (sand plus biomass) being utilized in the metal working fluid plant. One bioreactor was challenged with plant wastewater spiked with CMK at a concentration of 70 mg/L and a reactor was challenged without CMK, as control. The study was conducted for at least 6 weeks, with a hydraulic retention time similar to the one observed in the metal working plant treatment facility (18 h, 0.55 L/h). Microbial biomass was first adapted to the experimental conditions in the two reactors for 17 days: similar COD decrease was observed during this adaptation period in the two bioreactors (53±10% in the control and 56±11% in the second bioreactor). Following the addition of CMK to the second bioreactor, the bacteria did not exhibit any toxicity during the first week, with still similar COD biodegradation rate (55±6% in the control and 61±2% in the second bioreactor) and the active substance was removed more than 98.6% immediately upon feeding it to the bioreactor. An unexplained toxicity occurred then for two weeks in the bioreactor containing CMK, with a lower COD degradation rate (41±9%) compared to the control bioreactor (62±12%) and overall an important decrease in CMK biodegradation rate (<25%). A new supply with wastewater allowed restoring the COD biodegradation rate (70±6% compared to 65±10% in the control) and the CMK biodegradation rate (>98.6%) until the end of the experiment.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/11/11
Evaluation of applicant's justification	[redacted]
Conclusion	
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 7.1.2.1.1 Annex Point IIIA 12.2	Biological sewage treatment Aerobic biodegradation (11)
Reference	Bolz, U. et al., 1999: Determination of phenolic xenoestrogens in sediments and sewage sludges by HRGC/LRMS. Organohalogen Compounds, Vol. 40, 65-68. Bolz, U. et al., 2001: Phenolic xenoestrogens in surface water, sediments, and sewage sludge from Baden-Württemberg, south-west Germany. Environmental Pollution, 115, 291-301. Körner, W. et al., 2000: Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany. Chemosphere, Vol. 40, 1131-1142. Schnaak, W. et al., 1997: Organic contaminants in sewage sludge and their ecotoxicological significance in the agricultural utilization of sewage sludge. Chemosphere, Vol. 35, 5-11. Ternes, Th. A., 1998: Simultaneous determination of antiseptics and acidic drugs in sewage and river water. Vom Wasser, 90, 295-309. .
Studies summary	Bibliographical monitoring data are available for influent and effluents of sewage treatment plants, as well as for sewage sludge. The samplings have been carried out at different STPs, mainly in three different regions of Germany. The results are summarized below and in the following table. Concerning STP samplings, Schnaak et al. (1997) took sewage sludge samples from 25 wastewater treatment plants with different discharge (domestic, municipal, industrial), all located in Brandenburg, Germany. Analysis of the influents were not performed. CMK was found at *maximum* concentrations of approximately 0.1 and 0.6 mg/kg dry matter in summer and winter, respectively. The corresponding *median* values were approximately 0.02 and 0.004 mg/kg dry weight. Ternes et al. (1998) collected random samples of 49 German municipal STP effluents in order to analyse several antiseptics. Two sampling periods had been carried out, one in November 1995 and one in September 1996. In no effluent CMK could be detected above the LOD of 0.01 µg/L. Furthermore, Ternes et al. analysed daily 24 h composite samples from raw influent and effluent over 6 days in a municipal STP close to Frankfurt, Germany, in November 1996. In the 6-days average influent samples of the Frankfurt STP, CMK was detected at an average concentration of 1.5 µg/L, whereas the compound was no longer found in the effluent (LOD = 0.01 µg/L). Several analyses have been performed at the municipal STP Steinhäule (Ulm, Germany). This STP receives 60% of the wastewater from households and the rest from industries, hospitals, and research institutes. The main industrial activities in the catchment area belong to the metal working, electronics, food processing and pharmaceutical and production of plastic resins sectors. There is neither pulp and paper industry nor an refinery in the area. At first, influent and effluent samples of the STP Steinhäule were taken in March and June 1998 (Körner et al., 2000). For this purpose, influent samples were taken every 2 hours over a period of 24 hours and effluent samples were taken 8 hours later than influent samples, to accommodate the chemical's residence time in the STP The twelve 2h-samples were pooled to form a 24h-sample. The samplings were carried out in periods of dry weather, and dilution effects by rainwater had therefore not occurred. Influent concentrations accounted

Section 7.1.2.1.1 Annex Point IIIA 12.2	Biological sewage treatment Aerobic biodegradation (11)
	for 0.13 µg/L in March and 0.5 µg/L in June, and the respective effluent concentrations were < 0.008 µg/L and not detectable. Hence CMK was eliminated by >94% and 100%, respectively, during wastewater treatment. At the same period, Bolz et al. (1999) have analysed sewage sludge samples from the same STP. CMK was detected in the sewage sludge samples at concentrations of 0.04 mg/kg in March and 0.003 mg/kg dwt in July. Other analyses have been performed in the sludge of the Steinhäule STP (Bolz et al., 2001; Körner et al., 2001). The sludge samples had been taken on three different days in 1998 and 1999. At these sampling time, dry weather as well as rainy days dealing to elevated water levels have occurred and yet similar amounts of CMK than previously have been detected (14 – 40 µg/kg dwt). In summary, monitoring data support the argument that CMK is in a large extend degraded in the STP. Furthermore, data of sewage sludge samples show CMK contents of generally ≤0.04 mg/kg dwt.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15/06/12
Evaluation of applicant's justification	[redacted]
Conclusion	
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	

Table 1 .: **Monitoring data on the occurrence of CMK sewage treatment plants (in- and effluent, sewage sludge)**

Location	Sampling date	Nb of samples	LOD/LOQ	Value	Reference
STP influent/effluent					
Steinhäule (Ulm, Germany)	March 10/11, 1998 June 29/30, 1998	1 (composite of 12 samples) 1 (composite of 12 samples)	≤0.01 µg/L / n.r. ≤0.01 µg/L / n.r.	0.130 µg/L (influent) < 0.008 µg/L (effluent) 0.500 µg/L (influent) n.d. (effluent)	Körner et al., 2000
Several STP in Germany Frankfurt/Main (Germany)	Nov 1995, Sept, 1996 November 1996	82 6-day average samples	0.01 µg/L / n.r. 0.01 µg/L / n.r.	< LOD (effluent) 1.5 µg/L (±0.5, influent) < LOD (effluent)	Ternes et al, 1998
Sewage sludge					
Steinhäule (Ulm, Germany)	March 2, 1998 July 8, 1998	2	n.r.	0.04/0.003 mg/kg dwt	Bolz et al., 1999
Steinhäule (Ulm, Germany)	1998 and 1999	3	≤ 0.5 - 19 µg/kg dwt/ 0.6 – 35 µg/kg dwt	14 – 40 µg/kg dwt	Bolz et al., 2001
State of Brandenburg (Germany)	n.r.	Samples from 25 STPs	n.r.	Maxima: 0.1 mg/kg dwt (summer) 0.6 mg/kg dwt (winter) Median: 0.02 mg/kg dwt (summer) 0.004 mg/kg (winter)	Schnaak et al., 1997

n.r. = not reported

n.d. = not detected

Section 7.1.2.1.1 **Annex Point IIIA 12.2**	**Biological sewage treatment** **Aerobic biodegradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure […]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification […].**	
Detailed justification:	[redacted]	
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	May 2009	
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 A7.1.2.1.2 Anaerobic biodegradation (01)

Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Reis, K.-H. (2007): Anaerobic biodegradability of 4-chloro-3-methylphenol (Preventol CMK) in digested sludge: Measurement of gas production. Institut für biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Report No. 32321158, unpublished, Date: 2007-03-22	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline for Testing Chemicals - Proposal for a New Guideline 311. Anaerobic Biodegradation of Organic Compounds in Digested Sludge: Measurement of Gas Production; Revised Draft Document, October 2003. International Standard EN ISO 11734, Water quality – Evaluation of the ultimate biodegradability of organic compounds indigested sludge - Method by measurement of the biogas production, November 1998.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol, Preventol CMK)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Non-radiolabelled test substance	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Not relevant	
3.1.5	Composition of Product	Test with the active ingredient, therefore not relevant	
3.1.6	TS inhibitory to microorganisms	See Point 5.2	
3.1.7	Specific chemical analysis	No test item specific analysis was done	
3.2	**Reference substance**	Yes, sodium benzoate	

Section A7.1.2.1.2 Anaerobic biodegradation (01)

Annex Point IIIA XII 2.1

3.2.1	Initial concentration of reference substance	About 170 mg/L corresponding to about 99 mg organic carbon/L	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_2_1_2-1.	
3.3.2	Test system	The test system is described in Table A7_1_2_1_2-2.	
3.3.3	Test conditions	The test conditions are described in Table A7_1_2_1_2-3.	
3.3.4	Method of preparation of test solution	Appropriate amounts of test item and reference item were weighted directly into the test vessels. No emulsifiers or solvents were used for the test solution preparation. The preparation of the test flasks is described in Table A7_1_2_1_2-3.	
3.3.5	Initial TS concentration	About 170 mg/L corresponding to 99 mg organic carbon/L	X
3.3.6	Duration of the test	60 days	
3.3.7	Analytical parameter	Carbon dioxide and methane production	
3.3.8	Sampling	Continuous measurement: The measuring equipment recorded 360 readings per 60 days of running test.	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Controls	Background gas production: Inoculated mineral medium without test substance Toxicity control: Test item and reference item were added to a vessel containing the test medium, each at the concentration as added in the treatments, respectively.	
3.3.11	Statistics	Not relevant	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Degradation of TS in abiotic control	Not reported. The lack of degradation of the test substance during the course of the study reveals no abiotic degradation of the compound.	
4.1.2	Degradation	The anaerobic biodegradation of the test substance and the reference item is shown in Table A7_1_2_1_2-4. The Table also includes the results of the toxicity control.	
4.1.3	Graph	The results are presented in tabular form (Table A7_1_2_1_2-4)	
4.1.4	Other observations	None	
4.1.5	Degradation of reference substance	The anaerobic biodegradation of the reference item is shown in Table A7_1_2_1_2-4.	

Section A7.1.2.1.2 Anaerobic biodegradation (01)

Annex Point IIIA XII 2.1

4.1.6	Intermediates/ degradation products	Not identified (*cf.* Point 3.3.9)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The purpose of the test was to determine the anaerobic biodegradability of p-chloro-m-cresol in digested sludge from a municipal waste water treatment plant during 60 days. The anaerobically digesting sludge degrades biodegradable substances and gas is produced (methane plus carbon dioxide), which was measured by a manometric device. Based on the known maximum methane and carbon dioxide release of the test item and the produced biogas, the degradation rate of the test item was determined. The test was performed at conditions corresponding to the anaerobic stage of a waste water treatment plant. The study is based on international test guidelines.
5.2	**Results and discussion**	p-Chloro-m-cresol (Preventol CMK) was found not to be biodegradable under the anaerobic conditions of the test system. No net carbon-production (as methane and carbon dioxide) was found. The anaerobic biodegradation of the reference item sodium benzoate under test conditions was found to be 75% (mean) within 60 days of incubation, thus confirming the suitability of the used digesting sludge inoculum. In the toxicity control containing both, the test item and the reference item sodium benzoate, no biodegradation was found. Thus, the test item can be assumed to be inhibitory on the digesting sludge microorganisms.
5.3	**Conclusion**	Under the conditions of this test, p-chloro-m-cresol can be considered not to be anaerobically biodegradable. However, the compound was found to be inhibitory on the digesting sludge microorganisms, hence, a potential for anaerobic biodegradation when applied at lower dosages can not be rouled out.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_2_1_2-1: Inoculum / Test organisms

Criteria	**Details**
Nature	Anaerobically digested sludge
Species	Mixed population of microorganisms
Strain	Not relevant
Source/Sampling site	Domestic waste water treatment plant Darmstadt, Germany
Laboratory culture	No (see above)
Method of cultivation	Not relevant
Preparation of inoculum for exposure	The digesting sludge was collected from the digesting tank into collecting bottles (gas tight seals, made of butyl rubber) and transported to the laboratory in an insulated container to minimize temperature shock. The digesting sludge was processed immediately after sampling. The sludge was gently stirred and passed through a sieve (mesh size 1 mm^2) under a head space of nitrogen gas. The digesting sludge pH was 7.3 and not adjusted. The sieved digesting sludge was washed by centrifugation and the supernatant liquid phase was decanted. The solid material was re-suspended in deoxygenated test water under nitrogen gas and again centrifuged. This procedure was repeated twice. Finally, the digesting sludge was re-suspended in one litre of test water.An aliquot of the final sludge suspension was weighed, dried and the ratio of wet sludge to its dry weight was determined. Based on this ratio, calculated aliquots of washed sludge suspension, corresponding to 24.8 g dry material per litre were mixed with test water and then incubated under nitrogen gas until use for one day.
Pretreatment	An appropriate volume of sieved anaerobically digesting sludge was gently stirred in a bottle (1 L glass flask) while passing a stream of nitrogen through the headspace. No nutrients or substrates were added and the digested sludge was incubated at 35°C ± 2°C for one day. An aliquot of the digested sludge suspension was weighed, dried and dry weight was determined. The sludge dry matter was used as calculation base for the preparation of test vessels. According to the dry weight of 24.8 g/L, a volume of 30 mL digesting sludge suspension was used as inoculum for each test vessel..
Initial cell concentration	The resulting suspended solids concentration was 2.1 g/L and within the range of 1 to 3 g/L

Table A7_1_2_1_2-2: Test system

Criteria	Details
Culturing apparatus	Pressure-resistant, gas-tight glass bottles of approximately 640 mL total volume (i.e. 500 mL (nominal) GL 32 glass bottles with incorporated glass tubes for sampling) were used as test units. The bottles were gas-tight closed by the measuring device.
Number of replicates/concentration	Test item: Five replicates. Reference item / toxicity control: Three replicates. Control unit: Six replicates.
Measuring equipment	Measurement of the gas production: BSB Sensomat System, Aqualytic Langen Germany. The system consists of a measuring head (pressure sensor) and the scientific controller, which interacts via infrared radiation. The measuring head recorded 360 readings per time interval, e.g. 360 readings per 60 days when test is running. The stored pressure data were transferred via RS 232 cable to a PC and visualized using a standard PC-program (MS Excel). The produced biogas increased the pressure in the test bottle and the pressure sensor recorded the actual pressure at each reading. If the pressure is out of range (reference item treatments), a pressure equalisation of the test bottle was done by shortly opening the lid. At the end of the test, the inorganic carbon content of the test solution supernatant was determined by measuring the dissolved inorganic carbon (IC) as carbon dioxide.
Oxidation reduction indicator	Yes, resazurin

Table A7_1_2_1_2-3: Test conditions

Criteria	Details
Composition of medium	The following constituents were used for the preparation of the mineral medium: KH_2PO_4 0.27 g Na_2HPO_4 x 12 H_2O 1.12 g NH_4Cl 0.53 g $CaCl_2$ x 2 H_2O 0.075 g $MgCl_2$ x 6 H_2O 0.10 g $FeCl_2$ x 4 H_2O 0.02 g Resazurin 0.001 g Na_2S x 9 H_2O 0.20 g de-oxygenated deionised water add 1000 mL pH 7 ± 0.2
Additional substrate	None
Solvent	Appropriate amounts of test item and reference item were weighted directly into the test vessels. No emulsifiers or solvents were used for the test solutions preparation.
Preparation of medium	Directly before adding the inoculum, the test bottles containing the test item or the reference item were flushed with a stream of nitrogen gas for approximately 2 minutes to remove air. Aliquots of the well mixed digesting sludge suspension were dispensed in the test bottle a graduated measuring cylinder. While adding inoculum and substrate, the bottles were kept under a flow of nitrogen gas. Based on the total volume of 640 mL, each test flask contained 330 mL of test solution and 30 mL of digesting sludge suspension. The total free gas volume (headspace) was 280 mL (ratio test solution to headspace: about 44% : 56%).
Test temperature	30 - 37°C in the dark
pH	7.3 (digesting sludge suspension) 7.4 (test solutions, measured at the start of the test in separately prepared flasks)
Suspended solids concentration	The suspended solids concentration was 2.1 g/L and within the range of 1 to 3 g/L.
Other relevant criteria	None

Table A7_1_2_1_2-4: Cumulative carbon production in test flasks during the test period of 60 days

Treatment	Test substance concentration (mg/L)	Reference substance concentration (mg/L)	Carbon per vessel[1] (mg)	Produced carbon[2] (mg/60 d)	Inorganic carbon[3] (mg/24 h)	Sum of produced carbon (total mg)	Sum of produced carbon (net mg)[4]	Degradation (%)
Control 1	--	--	--	10.61	8.44	19.05	--	--
Control 2	--	--	--	11.27	7.01	18.28	--	--
Control 3	--	--	--	10.75	9.35	20.10	--	--
Control 4	--	--	--	11.92	7.66	19.58	--	--
Control 5	--	--	--	9.43	8.57	18.00	--	--
Control 6	--	--	--	9.57	7.40	16.97	--	--
Mean	--	--	--	*10.59*	*8.07*	*18.66*	--	--
Test sub. 1	171.67	--	36.46	8.24	6.88	15.12	-3.54	--
Test sub. 2	170.00	--	36.11	5.89	7.01	12.90	-5.76	--
Test sub. 3	168.89	--	35.87	6.55	7.14	13.69	-4.97	--
Test sub. 4	170.00	--	36.11	7.86	6.36	14.22	-4.44	--
Test sub. 5	170.83	--	36.29	5.63	7.01	12.64	-6.02	--
Mean	*170.28*	--	*36.17*	*6.83*	*6.88*	*13.71*	*-4.95*	--
Ref. sub. 1	--	170.56	35.61	35.37	12.72	48.09	29.43	83
Ref. sub. 2	--	170.83	35.67	34.45	12.85	47.30	28.64	80
Ref. sub. 3	--	169.44	35.38	29.74	10.65	40.39	21.73	61
Mean	--	*170.28*	*35.55*	*33.19*	*12.07*	*45.29*	*26.60*	*75*
Tox. 1	170.56	171.11	71.95	5.89	6.62	12.51	-6.15	--
Tox. 2	171.11	170.56	71.96	7.47	6.36	13.83	-4.83	--
Tox. 3	170.00	169.72	71.55	5.76	6.36	12.12	-6.54	--
Mean	*170.56*	--	*71.82*	*6.37*	*6.45*	*12.82*	*-5.84*	--

--: not applicable; Test sub. = Test substance (p-chloro-m-cresol);
Ref. sub= Reference substance (sodium benzoate); Tox. = Toxicity control
[1] Based on a carbon content of 0.59 mg/mg test item and 0.58 mg/mg reference item; volume of test vessel: 330 mgL
[2] Produced carbon in the test vessel over a period of 60 days
[3] Inorganic carbon in the test vessel released after acidification within 24 hours
[4] The produced carbon of the treatment is corrected by the produced carbon (mean) of the control

Section A7.1.2.1.2 Annex Point IIIA XII 2.1	**Anaerobic biodegradation (02_03_04)**
Reference	Voets JP, Pipyn P., van Lancker P, Verstraete W, 1976. Degradation of microbicides under different environmental conditions. .Journal of applied bacteriology, 40, 62-72 O'Connor OA and Young LY, 1989. Toxicityand anaerobic biodegradability of substituted phenols under methanogenic conditions. Environmental toxicology and chemistry. 8-853-862. Kirk PWW and Lester JN, 1989. Degradation of phenol, selected chlorophenols and chorophenoxy herbicides during anaerobic sludge digestion. Environmental technology letters, 10, 405-414
Studies summary	Voets et al. (1976) investigated the anaerobic degradation of CMK (20 mg/L) in a mineral solution inoculated with soil extract and with or without organic matter provided as synthetic sewage at 22°C. After 21 days of incubation, no degradation of the test substance could be observed for each tested medium. O'Conner and Young (1989) evaluated CMK for its anaerobic biodegradability and toxicity to methanogenesis using two anaerobic bioassays, the biochemical methane potential (BMP) and the anaerobic toxicity assay (ATA). Both parameters were used to evaluate the conversion of added substrate carbon to CO_2 and CH_4. CMK was added as the sole carbon source at concentrations of 20, 100 and 200 mg/L to a defined medium containing 10% (v/v) inoculum of municipal digester sludge. The incubation flasks were stored at 37°C in the dark. The addition of CMK as sole source of carbon to the inoculum did not produce methane in excess of the background control at any time during the incubation, indicating no significant biodegradation under anaerobic conditions. An additional toxicity test showed that the inhibition of methanogenesis occurred at all concentrations and increased with increasing concentrations of CMK. However, this inhibition was only temporarily, indicating an acclimatisation ability of the microorganisms. Kirk and Lester (1989) assessed the behaviour of CMK during anaerobic incubation of digested sludge (1.9% wt/vol total solids) under active and sterile conditions. The sludge was spiked with the compound at a concentration of 12.7 µg/L (corresponding to 7.5 µg organic carbon/L). The samples were sealed under an atmosphere of oxygen free nitrogen and placed in a water bath in the dark at 37°C for up to 32 days. A further set of samples was treated with 1 % (wt/vol) sodium azide to inhibit biological activity. CMK underwent a significant degradation during incubation in untreated and azide treated sludge. After 32 days of incubation, the test compound's concentration amounted to 10% of the initial concentration in the active sludge trials and approx. 50% of the initial concentration in azide sterile controls. Although a difference between untreated and azide treated sludge became obvious, it could not be confirmed by statistical means. However, it can be concluded that a combination of biotic and abiotic mechanisms may be responsible for the removal in the active sludge with only the abiotic mechanism being relevant in the sterile sludge.

Section A7.1.2.1.2 **Annex Point IIIA XII 2.1**	**Anaerobic biodegradation (02_03_04)**
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/11/11
Evaluation of applicant's justification	[redacted]
Conclusion	
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 A7.1.2.1.2 Anaerobic biodegradation (05)

Annex Point IIIA XII 2.1

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		1 REFERENCE
1.1	**Reference**	Feil, N. (2009): Anaerobic biodegradability of 4-Chloro-3-methylphenol (Preventol CMK) in digested sludge: Measurement of gas production. Institut für biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Report No. 45822168, unpublished, Date: 2009-05-18
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD Guideline for Testing Chemicals - Proposal for a New Guideline 311. Anaerobic Biodegradation of Organic Compounds in Digested Sludge: Measurement of Gas Production; Revised Draft Document, October 2003. International Standard EN ISO 11734, Water quality – Evaluation of the ultimate biodegradability of organic compounds indigested sludge - Method by measurement of the biogas production, November 1998.
2.2	**GLP**	Yes
2.3	**Deviations**	Additional flasks of the reference substance sodium benzoate were prepared with a higher concentration to ensure the biodegradation ability of the sludge.
		3 MATERIALS AND METHODS
3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Not relevant
3.1.5	Composition of Product	Test with the active ingredient, therefore not relevant
3.1.6	TS inhibitory to microorganisms	See Point 5.2
3.1.7	Specific chemical analysis	No test item specific analysis was done

Section A7.1.2.1.2 Anaerobic biodegradation (05)

Annex Point IIIA XII 2.1

3.2	**Reference substance**	Yes, sodium benzoate
3.2.1	Initial concentration of reference substance	About 80 mg/L, corresponding to about 47 mg organic carbon/L. A second concentration of sodium benzoate was also used to check the biodegradation ability of the sludge: About 160 mg/L, corresponding to about 93 mg organic carbon/L.
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_2_1_2-1.
3.3.2	Test system	The test system is described in Table A7_1_2_1_2-2.
3.3.3	Test conditions	The test conditions are described in Table A7_1_2_1_2-3.
3.3.4	Method of preparation of test solution	Appropriate amounts of test item and reference item were weighted directly into the test vessels. No emulsifiers or solvents were used for the test solution preparation. The preparation of the test flasks is described in Table A7_1_2_1_2-3.
3.3.5	Initial TS concentration	About 68 mg/L corresponding to 40 mg organic carbon/L
3.3.6	Duration of the test	60 days
3.3.7	Analytical parameter	Carbon dioxide and methane production
3.3.8	Sampling	Continuous measurement: The measuring equipment recorded 360 readings per 60 days of running test.
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Controls	Background gas production: Inoculated mineral medium without test substance Toxicity control: Test item and reference item were added to a vessel containing the test medium, each at the concentration as added in the treatments, respectively.
3.3.11	Statistics	Not relevant
	4 RESULTS	
4.1	**Degradation of test substance**	
4.1.1	Degradation of TS in abiotic control	Not reported. The lack of degradation of the test substance during the course of the study reveals no abiotic degradation of the compound.

Section A7.1.2.1.2 Anaerobic biodegradation (05)

Annex Point IIIA XII 2.1

4.1.2	Degradation	The test substance was found not to be biodegradable under the anaerobic conditions of the test system. No net carbon-production (as methane and carbon dioxide) was found. A pressure increase of the test item flasks appeared, but the gas production was low within 60 days of incubation. The anaerobic biodegradation of the test substance and the reference item is shown in Table A7_1_2_1_2-4. The Table also includes the results of the toxicity control.	
4.1.3	Graph	The results are presented in tabular form (Table A7_1_2_1_2-4)	
4.1.4	Other observations	None	
4.1.5	Degradation of reference substance	The anaerobic biodegradation of the reference item is shown in Table A7_1_2_1_2-4.	X
4.1.6	Intermediates/ degradation products	Not identified (*cf.* Point 3.3.9)	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of the test was to determine the anaerobic biodegradability of p-chloro-m-cresol in digested sludge from a municipal waste water treatment plant during 60 days. The anaerobically digesting sludge degrades biodegradable substances and gas is produced (methane plus carbon dioxide), which was measured by a manometric device. Based on the known maximum methane and carbon dioxide release of the test item and the produced biogas, the degradation rate of the test item was determined. The test was performed at conditions corresponding to the anaerobic stage of a waste water treatment plant.

The study is based on international test guidelines.

Section A7.1.2.1.2 Anaerobic biodegradation (05)

Annex Point IIIA XII 2.1

5.2	**Results and discussion**	p-Chloro-m-cresol (Preventol CMK) was found not to be biodegradable under the anaerobic conditions of the test system. No net carbon-production (as methane and carbon dioxide) was found. The anaerobic biodegradation of the reference item sodium benzoate under test conditions was found to be 15% (mean) within 60 days of incubation. Also no observable biodegradation of the second higher concentration of sodium benzoate (160 mg/L) was found. This is an effect of a very active sludge inoculum which contained much own carbon ressources.There was no need for the sludge bacteria to use other carbon sources Therefore a very high gas production in the blank controls appeared. In the toxicity control containing both, the test item and the reference item sodium benzoate, no biodegradation was found. The gas production was normal and almost similar to the reference item tests for the first part of the test period. In the second half of the test duration a slight inhibition of the gas production was observed. Thus, the test item can be assumed to be inhibitory on the digesting sludge microorganisms in small concentrations, therefore no biodegradation was possible.	X
5.3	**Conclusion**	Under the conditions of this test, p-chloro-m-cresol can be considered not to be anaerobically biodegradable.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	The test failed in part criteria of the OECD guideline 311, since in the second half of the test period the gas production was inhibited in the toxicity control flasks. Due to the high toxicity of CMK the test substance concentration has to be reduced whereas the sludge inoculum increased. Due to high activity of sludge inoculuma very high gas production appeared in the blank control and the sludge bacteria did not degrade the reference item.. The limited suitability of the test design for investigating anaerobic biodegradation of CMK is discussed in Appendix 2 of the test report (page 30 f.)	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2009
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_2_1_2-1: Inoculum / Test organisms

Criteria	Details
Nature	Anaerobically digested sludge
Species	Mixed population of microorganisms
Strain	Not relevant
Source/Sampling site	Domestic waste water treatment plant Darmstadt, Germany
Laboratory culture	No (see above)
Method of cultivation	Not relevant
Preparation of inoculum for exposure	The digesting sludge was collected from the digesting tank into collecting **PE-**bottles (gas tight seals~~, made of butyl rubber~~) and transported to the laboratory in an insulated container to minimize temperature shock. The digesting sludge was processed immediately after sampling. The sludge was gently stirred and passed through a sieve (mesh size 1 mm^2) under a head space of nitrogen gas. The digesting sludge pH was 7.3 and not adjusted. The sieved digesting sludge was washed by centrifugation and the supernatant liquid phase was decanted. The solid material was re-suspended in deoxygenated test water under nitrogen gas and again centrifuged. This procedure was repeated twice. Finally, the digesting sludge was re-suspended in ~~one litre~~ **1.5 L** of test water. ~~An aliquot of the final sludge suspension was weighed, dried and the ratio of wet sludge to its dry weight was determined. Based on this ratio, calculated aliquots of washed sludge suspension, corresponding to 2.7 g dry material per litre were mixed with test water and then incubated under nitrogen gas until use for one day.~~
Pretreatment	An appropriate volume of sieved anaerobically digesting sludge was gently stirred in a bottle ~~(1 L glass flask)~~ while passing a stream of nitrogen through the headspace. No nutrients or substrates were added ~~and the digested sludge was incubated at 35°C ± 2°C for one day~~. An aliquot of the digested sludge suspension was weighed, dried and dry weight was determined. The sludge dry matter was used as calculation base for the preparation of test vessels. According to the dry weight of 2.7 g/L, a volume of 70 mL digesting sludge suspension was used as inoculum for each test vessel..

Table A7_1_2_1_2-2: Test system X

Criteria	Details
Culturing apparatus	Pressure-resistant, gas-tight glass bottles of approximately 640 mL total volume (i.e. ~~500~~ **640** mL (nominal) GL 32 glass bottles with incorporated glass tubes for sampling) were used as test units. The bottles were gas-tight closed by the measuring device.
Number of replicates/concentration	Test item: ~~Five~~ **Four** replicates. Reference item **(80 mg/L):** **Three replicates** **Reference item (160 mg/L):** **Four replicates** **T**oxicity control: Three replicates. Control unit: Six replicates.
Measuring equipment	Measurement of the gas production: BSB Sensomat System, Aqualytic Langen Germany. The system consists of a measuring head (pressure sensor) and the scientific controller, which interacts via infrared radiation. The measuring head recorded 360 readings per time interval, e.g. 360 readings per 60 days when test is running. The stored pressure data were transferred via RS 232 cable to a PC and visualized using a standard PC-program (MS Excel). The produced biogas increased the pressure in the test bottle and the pressure sensor recorded the actual pressure at each reading. If the pressure is out of range (reference item treatments), a pressure equalisation of the test bottle was done by shortly opening the lid. At the end of the test, the inorganic carbon content of the test solution supernatant was determined by measuring the dissolved inorganic carbon (IC) as carbon dioxide.
Oxidation reduction indicator	Yes, resazurin

Table A7_1_2_1_2-3: Test conditions

Criteria	**Details**
Composition of medium	The following constituents were used for the preparation of the mineral medium: KH_2PO_4 0.27 g Na_2HPO_4 x 12 H_2O 1.12 g NH_4Cl 0.53 g $CaCl_2$ x 2 H_2O 0.075 g $MgCl_2$ x 6 H_2O 0.10 g $FeCl_2$ x 4 H_2O 0.02 g Resazurin 0.001 g Na_2S x 9 H_2O 0.20 g de-oxygenated deionised water add 1000 mL pH ~~7 ± 0.2~~ **7.2**
Additional substrate	None
Solvent	Appropriate amounts of test item and reference item were weighted directly into the test vessels. No emulsifiers or solvents were used for the test solutions preparation.
Preparation of medium	Directly before adding the inoculum, the test bottles containing the test item or the reference item were flushed with a stream of nitrogen gas for approximately ~~2~~ **3** minutes to remove air. Aliquots of the well mixed digesting sludge suspension were dispensed in the test bottle a graduated measuring cylinder. While adding inoculum and substrate, the bottles were kept under a flow of nitrogen gas. Based on the total volume of 640 mL, each test flask contained about 450 mL of test solution and a free gas volume (headspace) of about 190 mL (ratio test solution to headspace: about 70 : 30).
Test temperature	35 - 36°C in the dark
pH	7.3 (digesting sludge suspension) 7.2 (test solutions, measured at the start of the test in separately prepared flasks)
Other relevant criteria	None

Table A7_1_2_1_2-4: Cumulative carbon production in test flasks during the test period of 60 days

Treatment	Test substance concen-tration (mg/L)	Reference substance concentration (mg/L)	Carbon per vessel[1] (mg)	Produced carbon[2] (mg/60 d)	Inorganic carbon[3] (mg/24 h)	Sum of produced carbon (total mg)	Sum of produced carbon (net mg)[4]	Degradation (%)
Control 1	--	--	--	38.41	10.94	49.35	--	--
Control 4	--	--	--	38.86	12.89	51.75	--	--
Control 5	--	--	--	41.61	11.92	53.53	--	--
Control 6	--	--	--	41.97	13.07	55.04	--	--
Mean	--	--	--	*40.21*	*12.21*	*52.42*	--	--
Test sub. 1	68.67	--	18.20	29.26	9.75	39.01	-13.41	-74
Test sub. 2	68.44	--	18.14	30.62	10.80	41.42	-11.00	-61
Test sub. 3	68.00	--	18.02	29.99	10.82	40.81	-11.61	-64
Test sub. 4	68.89	--	18.26	32.07	10.57	42.64	-9.78	-54
Mean	*68.50*	--	*18.16*	*30.49*	*10.49*	*40.97*	*-11.45*	*-63*
Ref. sub. 3	--	79.78	20.93	38.80	9.66	48.46	~~7.04~~ **-3.96**	~~34~~ **-19**
Ref. sub. 4	--	80.67	21.16	42.87	9.85	52.72	0.30 2.29	1
Ref. sub. 5	--	80.89	21.22	44.14	10.57	54.71	~~*3.21*~~ ***-0.45***	11
Mean	--	*80.45*	*21.10*	*41.94*	*10.03*	*51.96*	-1.48	~~*15*~~ ***-2***
Ref. sub. 1*	--	159.33	41.80	42.08	8.86	50.94		-4
Ref. sub. 2*	--	159.33	41.80	38.31	9.52	47.83	-4.59	-11
Ref. sub. 4*	--	160.22	42.03	37.96	9.60	47.56	~~6.14~~ **-4.86**	~~15~~ **-12**
Ref. sub. 5*	--	160.44	42.09	44.10	7.88	51.98	-0.44	-1
Mean	--	*159.83*	*41.93*	*40.61*	*8.97*	*49.58*	~~*-0.09*~~ ***-2.84***	~~*0*~~ ***-7***
Tox. Contr. 2	68.44	81.11	39.42	32.44	7.29	39.73	-12.69	-32
Tox. Contr. 3	67.56	80.22	38.95	28.38	7.05	35.43	-16.99	-44
Tox. Contr. 5	67.56	80.67	39.07	39.84	11.47	51.31	-1.11	-3
Mean	*67.85*	*80.67*	*39.15*	*33.55*	*8.60*	*42.16*	*-10.26*	*-26*

--: not applicable;
Test sub. = Test substance (p-chloro-m-cresol);
Ref. sub = Reference substance (sodium benzoate); Tox. = Toxicity control
[1] Based on a carbon content of 0.59 mg/mg test item and 0.58 mg/mg reference item; volume of test vessel: 640 mgL
[2] Produced carbon in the test vessel over a period of 60 days
[3] Inorganic carbon in the test vessel released after acidification within 24 hours
[4] The produced carbon of the treatment is corrected by the produced carbon (mean) of the control
*** Reference Item Sodium Benzoate second concentration**

Section A7.1.2.1.2 Anaerobic biodegradation (06) – V1.1 including Amendment

Annex Point IIIA XII 2.1

Official use only

1 REFERENCE

1.1	**Reference**	Möndel, M. (2010): Anaerobic biodegradability of Preventol CMK in digested sludge. RLP AgroScience GmbH, Neustadt a. d. Weinstraße, Germany. Report No. AS 142, unpublished, Date: 2010-05-26 and Fent, G. (2010): Anaerobic biodegradability of Preventol CMK in digested sludge. RLP AgroScience GmbH, Neustadt a. d. Weinstraße, Germany. Final Report Amendment No.:1 (30.11.2010) regarding Report No. AS 142, unpublished, Date: 2010-05-26. [The change of study director from Möndel, M. to Feint, G. became necessary for preparation of the Final Report Amendment No. 1 since neither Möndel, M. nor Hein, W. (deputy) were available in the office at the relevant time.]
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD Guideline for Testing Chemicals - Proposal for a New Guideline 311. Anaerobic Biodegradation of Organic Compounds in Digested Sludge: Measurement of Gas Production; Revised Draft Document, October 2003. International Standard EN ISO 11734, Water quality – Evaluation of the ultimate biodegradability of organic compounds indigested sludge - Method by measurement of the biogas production, November 1998.
2.2	**GLP**	Yes
2.3	**Deviations**	~~None.~~ Yes: On study day 8 of the test period glucose was added to the test system. Due to an early shortcoming of carbon in the sludge, indicated by a decrease of active bacteria on day 8, it was decided to adjust the micro bacterial equilibration by addition of an external carbon source. With the additional carbon source (72 mg carbon from glucose) the anaerobic bacteria in the sludge were able to sustain the anaerobic degradation to the end of the test period of 50 days. The results of the biodegradation of the test item, reference item and inhibition control were not affected by the addition of the glucose because the biodegradation was assessed by calculation of the net carbon production. From each flask the total gasified carbon was calculated and the gasified carbon of the control was subtracted to get the net carbon production rate.

3 MATERIALS AND METHODS

Section A7.1.2.1.2 Anaerobic biodegradation (06) – V1.1 including Amendment

Annex Point IIIA XII 2.1

3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Not relevant
3.1.5	Composition of Product	Test with the active ingredient, therefore not relevant
3.1.6	TS inhibitory to microorganisms	See Point 5.2
3.1.7	Specific chemical analysis	No test item specific analysis was done
3.2	**Reference substance**	Yes, phenol (Batch no. [redacted])
3.2.1	Initial concentration of reference substance	104 mg/L corresponding to 80 mg organic carbon/L
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_2_1_2-1.
3.3.2	Test system	The test system is described in Table A7_1_2_1_2-2.
3.3.3	Test conditions	The test conditions are described in Table A7_1_2_1_2-3.
3.3.4	Method of preparation of test solution	Appropriate amounts of the test item and the reference item were weighted directly into the test vessels. No emulsifiers or solvents were used for the test solution preparation. The preparation of the test flasks is described in Table A7_1_2_1_2-3.
3.3.5	Initial TS concentration	About 136 mg/L corresponding to 80 mg organic carbon/L
3.3.6	Duration of the test	50 days
3.3.7	Analytical parameter	Carbon dioxide and methane production
3.3.8	Sampling	The measuring head recorded 360 readings per time interval. The first time interval was set for 15 days (one reading / hour), the second time interval was set for 35 days (8 readings / day). Afterwards the test was stopped since the degradation curve reached the plateau phase.
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Controls	Control: Inoculated mineral medium without addition of the test item

Section A7.1.2.1.2 Anaerobic biodegradation (06) – V1.1 including Amendment

Annex Point IIIA XII 2.1

		Reference control: Inoculated mineral medium containing the reference item phenol Inhibition control: Test item and reference item were added to a vessel containing the test medium, each at the concentration as added in the treatments, respectively. For a detailed description of the different treatments refer to Table A7_1_2_1_2-4.
3.3.11	Statistics	Not relevant
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Degradation of TS in abiotic control	Not reported. The lack of degradation of the test substance during the course of the study reveals no abiotic degradation of the compound.
4.1.2	Degradation	The anaerobic biodegradation of the test substance and the reference item is shown in the updated Table A7_1_2_1_2-5 (incl. corrections from the Amendment Report), which summarizes the results from the three different degradation periods (*cf.* Point 5.2). The Table also includes the results of the inhibition control.
4.1.3	Graph	The results are presented in tabular form (Table A7_1_2_1_2-5)
4.1.4	Other observations	None
4.1.5	Degradation of reference substance	The anaerobic biodegradation of the reference item is shown in Table A7_1_2_1_2-5 (incl. corrections from the Amendment Report).
4.1.6	Intermediates/ degradation products	Not identified (*cf.* Point 3.3.9)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The purpose of the test was to determine the anaerobic biodegradability of 4-chloro-3-phenol in digested sludge from a municipal waste water treatment plant during 50 days. The anaerobically digesting sludge degrades organic substances and biogas is produced (methane plus carbon dioxide), which was measured by a manometric device. Based on the known maximum methane and carbon dioxide release of the test item and the produced biogas, the degradation rate of the test item was determined. The test was performed at conditions corresponding to the anaerobic stage of a waste water treatment plant. The study is based on international test guidelines.

Section A7.1.2.1.2 Anaerobic biodegradation (06) – V1.1 including Amendment

Annex Point IIIA XII 2.1

5.2	**Results and discussion**	***Comment**: In the Final Report by Möndel an error occurred in the calculation of carbon in the system at day 8. In the study by Möndel the degradation rate at day 8 was subtracted from the mass of carbon at the beginning of the test (48 mg) instead of subtraction of the net carbon production in 8 days. This error as well as all subsequent errors (calculation of total mass of carbon in the system from day 8 to day 50 & calculation of degradation rate in this period) is corrected in this updated study summary in a transparent way: old values are crossed out, the corrected values and corrected or updated explanations are highlighted in green.* The calculation of the test results for percentage biodegradation was done in three steps. In the first period (day 0 to day 8) the degradation was calculated separately (*cf.* Table A7_1_2_1_2-6). At the beginning of the second period (day 8) glucose was added to all test vessels, thus the carbon content of the liquid phase was different from the first period and ~~the degradation was calculated for the incubation time from day 8 to day 50~~ the net produced carbon in 8 days was calculated and subtracted from the mass of carbon at the beginning of the test (*cf.* updated Table A7_1_2_1_2-7). At the end of the test period the inorganic carbon was measured and the percentage of degradation for this step was calculated (*cf.* Table A7_1_2_1_2-8). The total biodegradation was determined as the sum of the three single values (*cf.* updated Table A7_1_2_1_2-5). The test item 4-Chloro-3-methylphenol (Preventol CMK) was found not to be biodegradable under the anaerobic conditions of the test system. No net carbon-production (as methane and carbon dioxide) was found during the incubation time of 50 days. The gas production was even lower than in the control vessels (-3.5%). The total anaerobic biodegradation of the reference item phenol under test conditions was found to be ~~68.2%~~ 63.1% within 50 days of incubation, thus confirming the suitability of the used digesting sludge inoculum since the total biodegradation of phenol reached a plateau phase after day 40, and the degradation of more than 60% was reached at day 25. In the inhibition control containing both, the test item and the reference item phenol, a biodegradation of 26.8% was found for the entire study duration. The gas production was found to be less than in the test vessels containing only the reference item. Thus, the test item can be assumed to be inhibitory on the digesting sludge microorganisms.
5.3	**Conclusion**	Under the conditions of this test, 4-Chloro-3-methylphenol can be considered not to be anaerobically biodegradable. However, the compound was found to be inhibitory on the digesting sludge microorganisms, hence, a potential for anaerobic biodegradation when applied at lower dosages can not be rouled out.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	None

X

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	06/09/2011
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_2_1_2-1: Inoculum / Test organisms

Criteria	**Details**
Nature	Anaerobically digested sludge
Species	Mixed population of microorganisms
Strain	Not relevant
Source/Sampling site	Domestic waste water treatment plant ("Zentralklärwerk Lachen-Speyerdorf") in Neustadt an der Weinstraße, Germany
Laboratory culture	No (see above)
Method of cultivation	Not relevant
Preparation of inoculum for exposure	The digesting sludge was collected from the digesting tank into a 60 L-PE-barrel (sealed gas tight afterwards) and directly transported to the laboratory. The digesting sludge had a pH of 7.4. Before preparation of the inoculum the sludge was stored under cold conditions in a cooling chamber at 4°C for seven days. The test flasks with 1000 mL of total volume were prepared with about 600 mL of the test solution and a gas volume (headspace) of about 400 mL. The volumes used in the experiment were strongly dependent on digested sludge concentration (based on dry matter) and activity as well as on the solubility of the test item. Therefore, 15 L of the inoculum were mixed in a 20 L container under a stream of nitrogen gas as follows: Dilution water 10 L Dilution medium 1500 mL Na_2HPO_4 x 10 H_2O (16.8g/250 mL) 250 mL Digested sludge 1500 mL Resazurin (oxygen indicator, 1mg/mL) 1.5 mL Na_2S x 9 H_2O (1g/10 mL) 30 mL The vessels containing the above listed substances were rinsed with dilution water after emptying. The rinsate was added to the inoculum. Finally the inoculum was filled up to a final volume of 15 L with dilution water.
Pretreatment	An appropriate volume of anaerobically digesting sludge was gently stirred in a bottle while passing a stream of nitrogen through the headspace for 30 minutes. No nutrients or substrates were added. For reduction of background gas production and to decrease the influence of the blank controls, pre-digestion of the sludge was performed. The sludge was allowed to digest without the addition of any nutrients or substrates at 35°C ± 2°C for eight days. The pH-value of the sludge suspension was 7.7 after pre-incubation. Four aliquots of 100 g the digested sludge suspension were weighed, dried and dry weight was determined. The sludge dry matter (2.9% ± 0.05%) was used as calculation base for the preparation of test vessels.

Table A7_1_2_1_2-2: Test system

Criteria	Details
Culturing apparatus	Pressure-resistant, gas-tight glass bottles of 1000 mL total volume were used as test units. The bottles were gas-tight selaed by the measuring device (Oxitop).
Number of replicates/concentration	Control unit (inoculum without addition of the test item): Six replicates. Test item: Six replicates. Reference item (reference item phenol was added to the vessels at a concentration of 104 mg/L): Six replicates. Inhibition control (test item and reference item were added to the vessels; each at the concentration as added in the treatments, respectively): Six replicates.
Measuring equipment	Measurement of the gas production: Oxitorp System, WTW Germany. The system consists of a measuring head (pressure sensor) and the scientific controller, which interacts via infrared radiation. The measuring head recorded 360 readings per time interval. The first time interval was set for 15 days (one reading / hour), the second time interval was set for 35 days (8 readings / day). Afterwards the test was stopped since the degradation curve reached the plateau phase. During incubation time, the samples were stirred gently for about 1 minute (using a magnetic stirrer) once per day to get a homogenous suspension. The stored pressure data were transferred via RS 232 cable to a PC and visualized using a standard PC-program (MS Excel). The produced biogas increased the pressure in the test bottle and the pressure sensor recorded the actual pressure at each reading. The pressure could be recorded in the range ± 300 mbar by the Oxitorp system. If the pressure was at 250 mbar at daily control (reference item treatment and controls), a pressure equalisation of the test bottle was done by shortly opening the lid. At day 8 all test vessels were opened and in each vessel 300 mg/L Glucose corresponding to 120 mg Carbon/L was added. At the end of the test, the inorganic carbon content of the test solution supernatant was determined by measuring the dissolved inorganic carbon (IC) as carbon dioxide. Therefore, the pressure in each of the test vessels was adjusted to atmospheric pressure. The IC in the supernatant was determined after acidifying the liquid to approximately pH 1 by adding 3.5 mL of concentrated mineral acid (H_2SO_4 96%). The vessels were stirred and then incubated for about 24 hours at 35°C ± 2°C and the pressure, resulted from the evolved CO_2 was measured.
Oxidation reduction indicator	Yes, resazurin

Table A7_1_2_1_2-3: Test conditions

Criteria	Details
Preparation of test medium	Dilution water: The dilution water was prepared of deionised water which was deoxygenated prior to use by passing nitrogen gas through the container for about one hour to ensure complete oxygen-free conditions. The deoxygenated dilution water was used for all dilutions prepared during the study. Composition of the stock solution: The stock solution of trace elements was prepared in dilution water: $MnCl_2$ x 4 H_20 50.4 mg H_3BO_3 5.5 mg $ZnCl_2$ 6.2 mg $CuCl_2$ 3.2 mg Na_2MoO_4 x 2 H_20 1.1 mg $CoCl_2$ x 6 H_2O 100.2 mg $NiCl_2$ x 6 H_2O 9.9 mg Na_2SeO_3 6 mg Dilution water to 1000 mL Preparation of dilution medium: The dilution medium was prepared in 10-fold concentrated stock solution: KH_2PO_4 5.40 g NH_4Cl 10.60 g $CaCl_2$ x 2 H_2O 1.50 g $MgCl_2$ x 6 H_2O 2.00g $FeCl_2$ x 4 H_2O 0.40 g Stock solution of trace elements 200 mL Dilution water to 2000 mL Na_2HPO_4 was separately dissolved. Therefore, 16.8 g Na_2HPO_4 x 10 H_2O were weighed in and transferred into a 250 mL volumetric flask. The flask was filled with dilution water to the calibration mark.
Application procedure	The test item, the reference item and the inhibition control were weighed into the test vessels. Afterwards, the inoculum was added and the flasks were purged with a stream of nitrogen gas to remove air. The test systems allowed for the preincubation at 35°C ± 2°C for about 1 hour while stirring to solve the test and reference item completely. After temperature equilibration was achieved a pH value of 7.8 was measured in one flask of each treatment After initial weights were recorded all vessels were introduced into the incubation chambers. The recording of data (gas pressure and temperature) was started. Based on the total volume of 1000 mL, each test flask contained about 600 mL of test solution. The total free gas volume (headspace) was about 400 mL (ratio test solution to headspace: about 3:2).

Additional substrate	None
Solvent	Appropriate amounts of test item, the reference item and the inhibition control were weighted directly into the test vessels. No emulsifiers or solvents were used for the test solutions preparation.
Test temperature	35°C ± 2°C in the dark
Incubation duration	50 days
pH	7.4 (digesting sludge) 7.8 (test solutions, measured at the start of the test in one flask of each treatment)
Other relevant criteria	None

Table A7_1_2_1_2-4: Detailed preparation of the treatments

Treatment	Number of replicates	Test item concentration (mg/L)	Test item per flask (mg)	Reference item concentration (mg/L)	Reference item per flask (mg)
Control [1)]	6	--	--	--	--
Test item [2)]	6	136	81.6 ± 0.4	--	--
Reference item [3)]	6	--	--	104	62.4 ± 1.0
Inhibition control [4)]	6	136	81.6 ± 0.3	104	62.4 ± 1.0

[1)] Control: Inoculum, without addition of the test item

[2)] Test item: Appropriate amounts of the test item were weight directly into the test vessels. No emulsifiers or solvents were used for the test solution.

[3)] Reference item: Simultaneously to the study with the test item, the reference substance phenol was tested at the nominal test concentration of about 104 mg/L corresponding to about 80 mg/L organic carbon per litre. Since the test item was added directly to the vessels, the same procedure was applied with the reference item.

[4)] Inhibition control: In order to obtain information on the toxicity of the test item to anaerobic micro-organisms, test item and reference item was added to vessels, each at the concentration as added in the treatments, respectively.

Table A7_1_2_1_2-5: Summary of percentage biodegradation of test item, reference item and inhibition control (including the corrections from the Final Report Amendment No.1 – corrected values are marked green)

Treatment	Mass of carbon per vessel (mg)	Addition of Glucose (mg/L)	Mass of total carbon per vessel (mg)	Degradation from day 0 to day 8 (%)	Degradation from day 8 to day 50 (%)	Degradation of Glucose from day 8 to day 50 (%)	Degradation from IC 24 h (%)	**Total Degradation (%)**
Control 1	--	300	72	--	--	85.8	--	--
Control 2	--	300	72	--	--	90.7	--	--
Control 3	--	300	72	--	--	85.0	--	--
Control 4	--	300	72	--	--	85.5	--	--
Control 5	--	300	72	--	--	89.2	--	--
Control 6	--	300	72	--	--	85.8	--	--
Mean						*87.0*		
Test sub. 7	48	300	120	4.1	~~-9.6~~ -9.4	--	-4.1	~~-9.6~~ -9.4
Test sub. 8	48	300	120	2.5	~~-7.2~~ -7.1	--	-3.9	~~-8.6~~ -8.5
Test sub. 9	48	300	120	3.7	~~-9.2~~ -9.1	--	-0.5	~~-6.1~~ -5.9
Test sub. 10	48	300	120	3.7	-3.1	--	-2.5	~~-1.9~~ -1.8
Test sub. 11	48	300	120	8.0	~~-2.2~~ -2.1	--	-2.2	~~3.5~~ 3.6
Test sub. 12	48	300	120	6.0	~~1.8~~ 1.7	--	-7.0	0.8
Mean				*4.7*	-4.9		*-3.4*	*~~-3.6~~ -3.5*
Ref. sub. 13	48	300	120	12.3	~~35.4~~ 33.4	--	-1.1	46.6
Ref. sub. 14	48	300	120	10.7	~~29.9~~ 28.5	--	0.6	~~41.3~~ 39.8
Ref. sub. 15	48	300	120	16.2	~~47.2~~ 43.6	--	4.8	~~68.2~~ 64.6
Ref. sub. 16	48	300	120	18.1	~~54.5~~ 49.9	--	4.5	~~77.2~~ 72.6
Ref. sub. 17	48	300	120	24.4	~~54.0~~ 47.6	--	7.4	~~85.7~~ 79.4
Ref. sub. 18	48	300	120	24.0	~~54.5~~ 48.3	--	5.2	~~83.7~~ 77.4
Mean				*17.6*	*~~45.9~~ 41.9*		*3.6*	*~~67.1~~ 63.1*
Inhibit. 19	96	300	168	10.4	5.8	--	1.0	17.2
Inhibit. 20	96	300	168	12.2	10.4	--	0.9	23.5
Inhibit. 21	96	300	168	13.6	~~8.6~~ 8.5	--	2.6	24.7
Inhibit. 22	96	300	168	12.2	~~10.8~~ 10.7	--	1.6	24.6
Inhibit. 23	96	300	168	10.2	9.8	--	3.2	23.2
Inhibit. 24	96	300	168	20.6	~~18.4~~ 18.3	--	8.6	~~47.5~~ 47.4
Mean				*13.2*	*10.6*		*3.0*	*26.8*

--: not applicable; Test sub. = Test substance (4-chloro-3-phenol);
Ref. sub= Reference substance (phenol); Inhitbit. = Inhibition control

Table A7_1_2_1_2-6: Cumulative carbon production in test flasks during the test period of 8 days

Treatment	Number of replicates	Number of Flask	Test item concentration (mg/L)	Reference item concentration (mg/L)	Carbon concentration (mg/L)	Mass of carbon (mg)	Net production of carbon in 8 days (mg)	Degradation from day 0 to day 8 (%)
Control	6	1	--	--	--	--	--	--
Control		2	--	--	--	--	--	--
Control		3	--	--	--	--	--	--
Control		4	--	--	--	--	--	--
Control		5	--	--	--	--	--	--
Control		6	--	--	--	--	--	--
Mean								
Test sub.	6	7	136	--	80	48	1.97	4.1
Test sub.		8	136	--	80	48	1.22	2.5
Test sub.		9	136	--	80	48	1.78	3.7
Test sub.		10	136	--	80	48	1.78	3.7
Test sub.		11	136	--	80	48	3.84	8.0
Test sub.		12	136	--	80	48	2.90	6.0
Mean			*136*		*80*	*48*	*2.25*	*4.7*
Ref. sub.	6	13	--	104	80	48	5.90	12.3
Ref. sub.		14	--	104	80	48	5.15	10.7
Ref. sub.		15	--	104	80	48	7.77	16.2
Ref. sub.		16	--	104	80	48	8.70	18.1
Ref. sub.		17	--	104	80	48	11.70	24.4
Ref. sub.		18	--	104	80	48	11.51	24.0
Mean				*104*	*80*	*48*	*8.46*	*17.6*
Inhibition c.	6	19	136	104	160	96	10.02	10.4
Inhibition c.		20	136	104	160	96	11.70	12.2
Inhibition c.		21	136	104	160	96	13.01	13.6
Inhibition c.		22	136	104	160	96	11.70	12.2
Inhibition c.		23	136	104	160	96	9.83	10.2
Inhibition c.		24	136	104	160	96	19.75	20.6
Mean			*136*	*104*	*160*	*96*	*12.67*	*13.2*

--: not applicable; Test sub. = Test substance (4-chloro-3-phenol);
Ref. sub= Reference substance (phenol); Inhitbition c. = Inhibition control

Table A7_1_2_1_2-7: Cumulative carbon production in test flasks during the test period of day eight to day 50 (including the corrections from the Final Report Amendment No.1 – corrected values are marked green)

Treatment	Number of replicates	Number of Flask	Addition of Glucose (mg/L)	Mass of carbon from Glucose (mg)	Mass of carbon at day 8 (mg)	Total mass of carbon at day 8 (mg)	Net produced carbon from day 8 to 50 (mg)	Degradation from day 8 to day 50 (%)
Control	6	1	300	72	0.0	72.0	--	--
Control		2	300	72	0.0	72.0	--	--
Control		3	300	72	0.0	72.0	--	--
Control		4	300	72	0.0	72.0	--	--
Control		5	300	72	0.0	72.0	--	--
Control		6	300	72	0.0	72.0	--	--
Mean								
Test sub.	6	7	300	72	~~43.9~~ 46.0	~~115.9~~ 118.0	-11.1	~~-9.6~~ -9.4
Test sub.		8	300	72	~~45.5~~ 46.8	~~117.5~~ 118.8	-8.5	~~-7.2~~ -7.1
Test sub.		9	300	72	~~44.3~~ 46.2	~~116.3~~ 118.2	-10.7	~~-9.2~~ -9.1
Test sub.		10	300	72	~~44.3~~ 46.2	~~116.3~~ 118.2	-3.6	-3.1
Test sub.		11	300	72	~~40.0~~ 44.2	~~112.0~~ 116.2	-2.5	~~-2.2~~ -2.1
Test sub.		12	300	72	~~42.0~~ 45.1	~~114.0~~ 117.1	2.0	~~1.8~~ 1.7
Mean					~~43.3~~ *45.8*	~~115.3~~ *117.8*	*-5.7*	*-4.9*
Ref. sub.	6	13	300	72	~~35.7~~ 42.1	~~107.7~~ 114.1	38.1	~~35.4~~ 33.4
Ref. sub.		14	300	72	~~37.3~~ 42.9	~~109.3~~ 114.9	32.7	~~29.9~~ 28.5
Ref. sub.		15	300	72	~~31.8~~ 40.2	~~103.8~~ 112.2	49.0	~~47.2~~ 43.6
Ref. sub.		16	300	72	~~29.9~~ 39.3	~~101.9~~ 111.3	55.5	~~54.5~~ 49.9
Ref. sub.		17	300	72	~~23.6~~ 36.3	~~95.6~~ 108.3	51.6	~~54.0~~ 47.6
Ref. sub.		18	300	72	~~30.4~~ 36.5	~~102.4~~ 108.5	52.4	~~54.5~~ 48.3
Mean					~~31.4~~ *39.5*	~~103.4~~ *111.5*	*46.6*	~~45.9~~ *41.9*
Inhibition c.	6	19	300	72	~~85.6~~ 86.0	~~157.6~~ 158.0	9.1	5.8
Inhibition c.		20	300	72	~~83.8~~ 84.3	~~155.8~~ 156.3	16.2	10.4
Inhibition c.		21	300	72	~~82.4~~ 83.0	~~154.4~~ 155.0	13.2	~~8.6~~ 8.5
Inhibition c.		22	300	72	~~83.8~~ 84.3	~~155.8~~ 156.3	16.8	~~10.8~~ 10.7
Inhibition c.		23	300	72	~~85.8~~ 86.2	~~157.8~~ 158.2	15.5	9.8
Inhibition c.		24	300	72	~~82.8~~ 76.3	~~154.8~~ 148.3	27.1	~~17.5~~ 18.3
Mean					~~*84.0*~~ *83.3*	~~*156.0*~~ *155.3*	*16.3*	~~*10.5*~~ *10.6*

--: not applicable; Test sub. = Test substance (4-chloro-3-phenol);
Ref. sub= Reference substance (phenol); Inhitbition c. = Inhibition control

Table A7_1_2_1_2-8: Inorganic Carbon Production in test flasks in 24 hours

Treatment	Number of replicates	Number of Flask	Total mass of carbon (mg)	Δ p acidification (hPa)	m_c control (mg)	m_{ic} Treatment (mg)	m_i inorganic carbon (mg)	Degradation from IC 24h (%)
Control	6	1	72.0	174.0	32.6	--	--	--
Control		2	72.0	176.0	32.9	--	--	--
Control		3	72.0	169.0	31.6	--	--	--
Control		4	72.0	165.0	30.9	--	--	--
Control		5	72.0	180.0	33.7	--	--	--
Control		6	72.0	164.0	30.7	--	--	--
Mean			*72.0*	*171.3*	*32.1*	--	--	--
Test sub.	6	7	115.9	146.0	--	27.3	-4.7	-9.6
Test sub.		8	117.5	147.0	--	27.5	-4.6	-7.2
Test sub.		9	116.3	168.0	--	31.4	-0.6	-9.2
Test sub.		10	116.3	156.0	--	29.2	-2.9	-3.1
Test sub.		11	112.0	158.0	--	29.6	-2.5	-2.2
Test sub.		12	114.0	129.0	--	24.1	-7.9	1.8
Mean			*115.3*	*150.7*	--	*28.2*	*-3.9*	*-4.9*
Ref. sub.	6	13	107.7	175.0	--	32.8	-1.2	-1.1
Ref. sub.		14	109.3	185.0	--	34.6	0.7	0.6
Ref. sub.		15	103.8	198.0	--	37.1	5.0	4.8
Ref. sub.		16	101.9	196.0	--	36.7	4.6	4.5
Ref. sub.		17	95.6	209.0	--	39.1	7.1	7.4
Ref. sub.		18	102.4	198.0	--	37.1	5.0	5.2
Mean			*103.4*	*193.5*	--	*36.2*	*3.5*	*3.6*
Inhibition c.	6	19	157.6	180.0	--	157.6	1.0	1.6
Inhibition c.		20	155.8	179.0	--	155.8	0.9	1.4
Inhibition c.		21	154.4	193.0	--	154.4	2.6	4.1
Inhibition c.		22	155.8	185.0	--	155.8	1.6	2.6
Inhibition c.		23	157.8	198.0	--	157.8	3.2	5.0
Inhibition c.		24	154.8	239.0	--	147.4	8.6	12.7
Mean			*156.0*	*195.7*	--	*154.8*	*3.0*	*4.6*

--: not applicable; Test sub. = Test substance (4-chloro-3-phenol);
Ref. sub= Reference substance (phenol); Inhitbition c. = Inhibition control

Section 7.1.2.1.2 Anaerobic biodegradation (07)

Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Gerharz, T. (2011): Degradation of 4-chloro-3-cresol in pork liquid manure under anaerobic conditions. Lanxess Deutschland GmbH, Leverkusen, Germany. Report No. D 2011-10, date: 2011-05-26 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LANXESS Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No; Internal test procedure developed for this experiment	
2.2	**GLP**	No	
2.3	**Deviations**	---	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Preventol® CMK	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	---	
3.1.5	Method of analysis	HPLC-UV	
3.2	**Reference substance**	Sodium benzoate: [redacted]	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Inoculum population existing in the sample of manure	
3.3.2	Test system	The experiment was carried out under argon air flow to exclude oxygen. To set up anaerobic conditions 100 ml - glass bottles were rinsed with argon. The liquid manure sample was mixed well and aliquoted to 80 g each (= 100 ml) into glass bottles. A one percent CMK solution in isopropanole was used. The CMK-free liquid manure was spiked with 3 mg / kg CMK. The added volume was maximal 100 µl / 100 ml. The samples were mixed well and the initial CMK content was determined by HPLC. Afterwards the bottles were closed with a septum and covered with argon by a cannula. In order to have a pressure balance for gas developing, e.g. methane gas in the liquid manure, a balloon filled with	X

Section 7.1.2.1.2 Anaerobic biodegradation (07)

Annex Point IIIA XII 2.1

		argon was fastened to a cannula and the cannula was stung through the septum. All tests were performed in duplicate. Details on the examined manure are given in **Table A7.1.2.1.2-1**. In order to prove degrading activity, sodium-benzoate is used as control substance. At each point of measurement the septum was removed and well mixed samples were taken with a one-way pipette. Subsequently, the bottles were locked again with the septum and covered with argon, in order to keep up the anaerobic environment. For the analysis of the CMK in the liquid manure approx. 4 g liquid manure was given in a 20 ml measuring flask. 2 ml 0.5 molecular sulphuric acid was added and afterwards filled up with THF to the calibration mark. This sample was put for approx. 30 min in the ultrasonic bath and after cooling centrifuged (14000 g, 7 min). The supernatant is used for HPLC analysis.	
3.3.3	Test conditions	Test was carried out under argon air flow to exclude oxygen.	
3.3.4	Method of preparation of test solution	For control reasons the fresh manure received from [redacted] was tested for presence of CMK. In the used liquid manure sample 4-chloro-3-cresol (CMK) was undetectable by HPLC. Test and Reference Item: A one percent CMK solution in isopropanole was used. Appropriate amounts of test item and reference item solution were weight directly into the glass bottle.	
3.3.5	Initial TS concentration	Test item p-Chloro-m-cresol: 3 mg CMK/ kg Reference item sodium benzoate: 100 mg sodium benzoate / kg	
3.3.6	Duration of the test	34 days	
3.3.7	Analytical parameter	CMK-removal	
		4 RESULTS	X
4.1	**Anaerobic degradation in manure**	In the test period of 34 days 4-chloro-3-cresol degraded with a half-life of 15 days under anaerobic conditions. Sodium benzoate was used as a control substance. It also degraded rapidly in a test period of 10 days with a half-life of 3 days **Table A7.1.2.1.2-2** and **Figure A7.1.2.1.2-1 and A7.1.2.1.2-2** summarise the degradation of CMK and the control substance in liquid manure	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Pork liquid manure was spiked with a 4-chloro-3-cresol containing solution. Sodium benzoate was used as a control substance. Degradation	

Section 7.1.2.1.2 Anaerobic biodegradation (07)

Annex Point IIIA XII 2.1

		of CMK was observed over a test period of 34 days. Test was carried out under argon air flow to exclude oxygen.
5.2	**Results and discussion**	In the test period of 34 days 4-chloro-3-cresol degraded with a half-life of 15 days under anaerobic conditions. Sodium benzoate was used as a control substance. It also degraded rapidly in a test period of 10 days with a half-life of 3 days.
5.3	**Conclusion**	4-chloro-3-cresol degrades in pork liquid manure with a half-life of 15 days under anaerobic conditions.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*22/10/2011*
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 A7.1.2.1.2-1: Examined manure

Origin	The tested manure is from a sow stable which is not disinfected.
Sample received from	[redacted]
Charge no.	[redacted]
pH	7-8

Table A7.1.2.1.2-2: Results of CMK and sodium-benzoate in liquid manure

Time [days]	bottle 1 [mg CMK/kg manure]	bottle 2 [mg CMK/kg manure]	arithmetic average +/- standard deviation
CMK			
0	2.96	2.87	2.92 ± 0.06
20	1.86	1.60	1.73 ± 0.18
27	0.34	0.97	0.66 ± 0.45
34	0.42	0.55	0.49 ± 0.09
Sodium-benzoate			
0	100.00	100.00	100.00 ± 0
5	46.00	21.00	33.50 ± 17.7
20	<0.20	0.20	0.20 ± 0
27	<0.20	<0.20	< 0.20 ± 0

Figure A7.1.2.1.2-1: Diagram on degradation of 4-chloro-3-methylphenol in liquid manure.

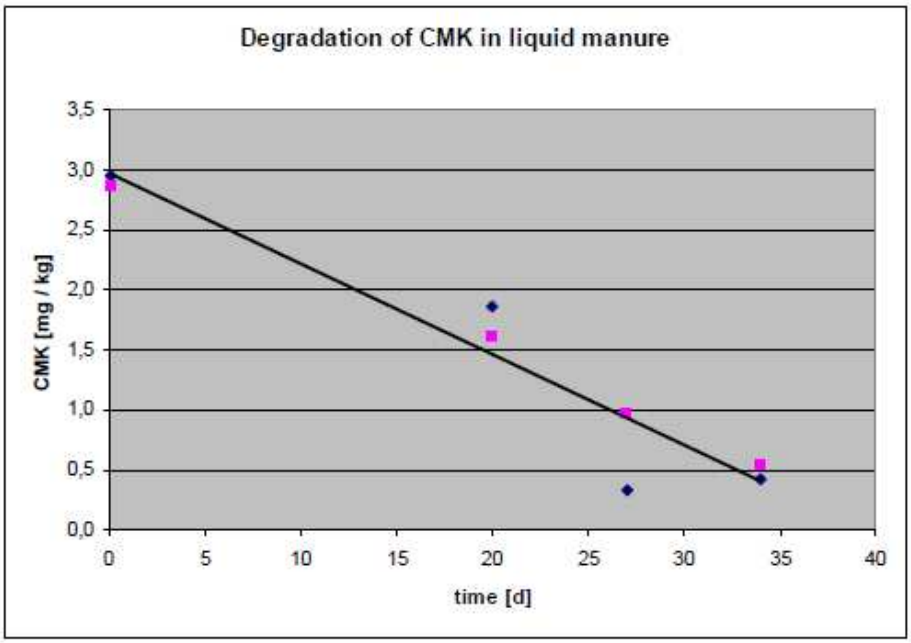
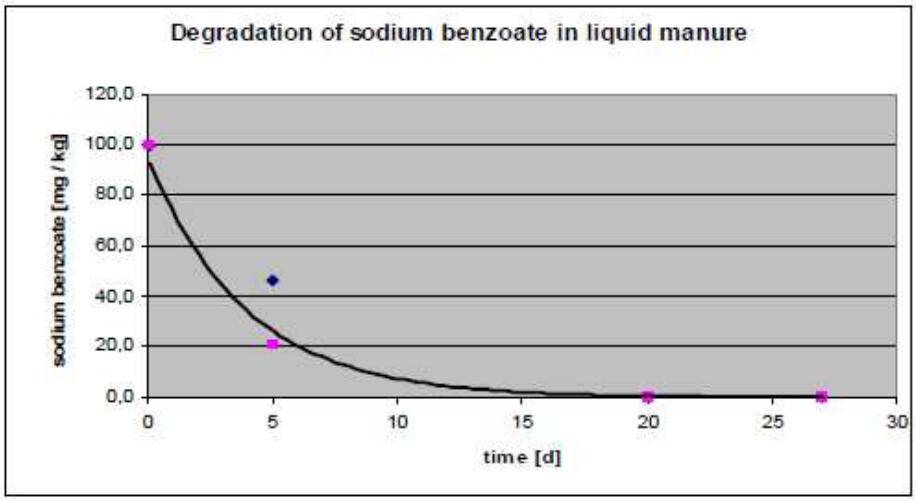


Figure A7.1.2.1.2-2: Diagram on degradation of the control substance in liquid manure.



Section A7.1.2.2.1 Aerobic aquatic degradation study (01)

Annex Point IIIA, 2.2.1

Official use only

		1 REFERENCE
1.1	**Reference**	Rast, H.-G. & Kölbl, H. (1987): Microbial degradation of Preventol CMK in Rhine water Bayer AG, FBT Leverkusen, Germany, Report No. LEV 14/76 and LEV 11/76, unpublished, Date: 1987-10-20
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Non-radiolabelled test substance 3-methyl-4-chlorophenol (p-chloro-m-cresol, 4-chloro-3-methylphenol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	None
3.2	**Reference substance**	None The mineralization of various phenol and pyrocatechol derivatives by two bacteria strains (cultured with the test compound as sole carbon source) was compared to that of p-chloro-m-cresol: Phenol, 2-methylphenol, 3-methylphenol, 2-chlorophenol, 3-chlororphenol, 4-chlorophenol, 2-methyl-4-chlorophenol, 5-methyl-2-chlorophenol, pyrocatechol, 4-methylpyrocatechol, 4-chloropyrocatechol, 3,5-dichloropyrocatechol, 3-methyl-4-chloropyrocatechol
3.2.1	Initial concentration of reference substance	Not relevant (see Point 3.2)
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_2_2_1-1.
3.3.2	Test system	The test system is described in Table A7_1_2_2_1-2.

Section A7.1.2.2.1 Aerobic aquatic degradation study (01)

Annex Point IIIA, 2.2.1

3.3.3	Test conditions	The test conditions are described in Table A7_1_2_2_1-3.	X
3.3.4	Method of preparation of test solution	p-Chloro-m-cresol was added as concentrated solution (20 g/L in NaOH)	
3.3.5	Initial TS concentration	Degradation in Rhine water: 100 mg/L and 200 mg/L p-Chloro-m-cresol degradation: 100 mg/L and 200 mg/L Degradation of phenol and pyrocatechol derivatives: 100 mg/L	
3.3.6	Duration of the test	Rhine water degradation test: 28 days p-Chloro-m-cresol degradation: approx. 48 hours Degradation of phenol and pyrocatechol derivatives: According to the nature of the test 30 min at maximum	
3.3.7	Analytical parameter	Rhine water degradation test: Measurement of p-chloro-m-cresol concentration p-Chloro-m-cresol degradation: Measurement of chloride concentration Degradation of phenol and pyrocatechol derivatives: Measurement of O_2 content	
3.3.8	Sampling	Rhine water degradation test: 0, 3, 7, 14, 28 days p-Chloro-m-cresol degradation: Approx. 0, 10, 24, 30, 48 hours Degradation of phenol and pyrocatechol derivatives: Continuous measurement	
3.3.9	Analysis	Rhine water degradation test: For the residue analysis a 2 mL sample of Rhine water was taken, acidified with concentrated phosphoric acid, mixed with 2 mL methanol and contrifuged, all under steile conditions, after which the residue was used for HPLC analysis. The measuring equipment is described in Table 7_1_2_2_1-2 p-Chloro-m-cresol degradation: See Table 7_1_2_2_1-2 Degradation of phenol and pyrocatechol derivatives: See Table 7_1_2_2_1-2	
3.3.10	Intermediates/ degradation products	Not identified	
3.3.11	Nitrate/nitrite measurement	Not applicable	

Section A7.1.2.2.1 Aerobic aquatic degradation study (01)

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3.3.12	Controls	Rhine water degradation test: Two sterile solutions were used as controls p-Chloro-m-cresol degradation: Uninoculated flasks were used as controls Degradation of phenol and pyrocatechol derivatives: DMSO control is not explicitly mentioned, however, O_2 measurement was conducted before and after the addition of phenols and catechols.
3.3.13	Statistics	Not relevant
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	The results of the p-chloro-m-cresol degradation in Rhine water are presented in Figures A7_1_2_2_1-1 and A7_1_2_2_1-2 The results of the p-chloro-m-cresol degradation by two bacterium strains (RST 160-1 and RST 160-2) isolated from Rhine water are presented in Figures A7_1_2_2_1-3 and A7_1_2_2_1-4 The degradation of phenol and pyrocatechol derivatives is presented in tabular form (Table A7_1_2_2_1-4)
4.1.2	Degradation	The results of the p-chloro-m-cresol degradation in Rhine water are presented in Figures A7_1_2_2_1-1 and A7_1_2_2_1-2 The results of the p-chloro-m-cresol degradation by two bacterium strains (RST 160-1 and RST 160-2) isolated from Rhine water are presented in Figures A7_1_2_2_1-3 and A7_1_2_2_1-4 The degradation of phenol and pyrocatechol derivatives is presented in Table A7_1_2_2_1-4
4.1.3	Other observations	None
4.1.4	Degradation of TS in abiotic control	A degradation of CMK in the sterile controls could not be observed (*cf.* Figures A7_1_2_2_1-1 to A7_1_2_2_1-4).
4.1.5	Degradation of reference substance	Not relevant (*cf.* Point 3.2)
4.1.6	Intermediates/ degradation products	Not relevant (*cf.* Point 3.3.10)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The microbial degradation of p-chloro-m-cresol at two different concentrations (100 and 200 mg/L) was investigated in Rhine water. Two bacteria strains (RST 160-1 and 160-2) were isolated from each of the two concentrations by culturing with p-chloro-m-cresol as the sole carbon source. Strain 160-1 was cultured with p-chloro-m-cresol on a larger scale and the mineralisation of various phenol and pyrocatechol derivatives was determined.

Section A7.1.2.2.1 Aerobic aquatic degradation study (01)

Annex Point IIIA, 2.2.1

5.2	**Results and discussion**	When p-chloro-m-cresol was added to Rhine water, complete degradation in all solutions containing it at a concentration of 100 mg/L was found after 7 to 14 days. At 200 mg/L complete degradation was found after 28 days in two of the four flasks. In the other two flasks a degradation of approximately 10% and 50% was observed after four weeks. Due to the high chloride content of the Rhine water an analytical balancing of the chloride formation in these enrichment cultures was not possible.	
		Two pure cultures (RST 160-1 and 160-2) were therefore isolated from each of the different initial concentrations which had led to a complete degradation. Both strains were able to grow with p-chloro-m-cresol as sole source of carbon. With these pure cultures the degradation of the test substance was measured in a mineral nutrient solution with a chloride content of 0.6 mmol/L. An equimolar quantitiy of inorganic chloride was released with the degradation of p-chloro-m-cresol. The two strains differed slightly in their degradation behaviour. When the concentration of the test compound was low, only slight growth was measured. After 48 hours a slight brown colouration was seen in both cultures, which is indicative of the elimination of oxidizable intermediates, presumably pyrocatechol derivatives.	X X
		The degradation route of p-chloro-m-cresol in freshwater was further investigated by culturing strain 160-1 with the test substance as sole carbon source and determining the mineralization of various phenol derivatives and pyrocatechol derivatives. The results reveal that phenol, various cresols and chlorocresols were oxidized, whereas among the chlorophenol isomers only 4-chlorophenol was oxidized, however, at a very low rate. Pyrocatechol, 4-methylpyrocatechol and 4-chloropyrocatechol were reacted at rates 10 to 15 times higher. The presumed intermediate of the degradation of p-chloro-m-cresol, i.e., 3-methyl-4-chloropyrocatechol, was oxidised to a high extent and had a considerable higher oxidation rate than the test compound.	
5.3	**Conclusion**	The test substance p-chloro-m-cresol is degradable by microorganisms in Rhine water within a few days and without lag-phase. At higher concentrations the degradation is slower however, these concentrations are unlikely to occur under natural conditions.	
		Isolated bacteria from the mixed cultures present in the Rhine water were able to grow using the test substance as the sole carbon source. The test substance was quantitatively degraded and dechlorinated.	
		An isolated CMK adapted bacteria strain was able to oxidise phenols, various cresols and chlorocresols whereas chlorophenols were not oxidized or degraded to a limited extent, only.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	Missing: Specification of the test material (purity, batch number), sampling and controls of the phenol degradation test, the degradation of the test substance in an abiotic control, test conditions (composition of the mineral medium, pH of the Rhine water degradation test, concentration of inoculum in the culture tests)	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2011
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_2_2_1-1: Inoculum

Criteria	Details
Rhine water degradation test	
Nature	Rhine water
Species	Mixed population of aquatic microorganisms
Source/Sampling site	Leverkusen-Opladen, 700 km
Initial cell concentration	Number of microorganisms in Rhine water: 1 x 10^5/mL
Preparation of inoculum for exposure	The Rhine water was distributed among 8 sterile 1 L Erlenmeyer flasks in portions of 250 mL
Pretreatment	None
Culture test	
Nature	p-Chloro-m-cresol degradation: Two cultures (RST 160-1 and 160-2) were isolated from each of the different initial concentrations of the Rhine water degradation test, which had led to complete degradation Degradation of phenol and pyrocatechol derivatives: Culture of strain 160-1, nature as described above
Preparation of inoculum for exposure	p-Chloro-m-cresol degradation: Transinoculation to sterile mineral nutrient solution containing p-chloro-m-cresol as the sole source of carbon: The cells were cultured in 25 mL standard I with 100 mg/L chlorophenol. After 24 hours the cells were contrifuged off under sterile conditions, washed with 0.05 M phosphate buffer and transinoculated into 250 mL mineral nutrient salt solution in 1 L Erlenmeyer flasks conaining p-chloro-m-cresol as the sole carbon source. Degradation of phenol and pyrocatechol derivatives: Strain 160-1 was cultured with p-chloro-m-cresol on a large scale: strain 160-1 was cultured in mineral medium in a 10-L brown fermentation flask with p-chloro-m-cresol as sole source of carbon. The growth substrate was added continuously as a 2% alkaline solution in such a way that a concentration of 100 mg/L was not exceeded. The cells were harvested at a cell density of about 1 g dried solids/L, washed with 0.05 M phosphate buffer and used for O_2 consumption or frozen.
Species	p-Chloro-m-cresol degradation: The pure cultures (RST 160-1 and 160-2) were mobile gram-negative bacteria that were not exactly identified. Degradation of phenol and pyrocatechol derivatives: Strain 160-1

Table A7_1_2_2_1-2: Test system

Criteria	Details
Rhine water degradation test	
Incubation apparatus	Sterile1-L Erlenmeyer flasks
Number of culture flasks/concentration	8 flasks Test concentrations: 100 mg/L and 200 mg/L
Aeration device	Shaking on a shaking machine at 220 rev./minute
Measuring equipment	HPLC analysis: Detector: UV Column: RP 8 Mobile phase: acetonitrile/water = 4/6 Limit of detection: 0.5 mg/L
Culture test	
Incubation apparatus	p-Chloro-m-cresol degradation: 1 L Erlenmeyer flasks Degradation of phenol and pyrocatechol derivatives: Culturing of strain 160-1 was done in a 10-L brown fermentation flask
Number of culture flasks/concentration	Not reported
Aeration device	p-Chloro-m-cresol degradation: Shaking on a shaking machine at 220 rev./minute
Measuring/measuring equipment	p-Chloro-m-cresol degradation: Cloride was determined with an Orion chloride electrode Degradation of phenol and pyrocatechol derivatives: The O_2 uptake rates were measured in 100 mL air saturated 0.1 M phosphate buffer, pH 7.3, at 28°C. The cells were suspended in the buffer at a cell density of 0.1 g dried solids/L and 100 µL of a 1% DMSO solution was added to the test substrate. The O_2 content in the solution was determined with an Orion oxygen electrode in a closed and temperature controlled vessel.

Table A7_1_2_2_1-3: Test conditions X

Criteria	Details
Rhine water degradation test	
Composition of medium	A mineral salt solution was added to the Rhine water. The composition is given in an extra report.
Additional substrate	None
Test temperature	28°C
pH	Not reported
Shaking	220 rev./minute
Concentration of inoculum	Number of microorganisms in the Rhine water: 1 x 10^5/mL
Other relevant criteria	None
Culture test	
Composition of medium	p-Chloro-m-cresol degradation: A mineral salt solution was added to the Rhine water. The composition is given in an extra report. Degradation of phenol and pyrocatechol derivatives: Air-saturated 0.1 M phosphate buffer
Additional substrate	None
Test temperature	p-Chloro-m-cresol degradation: 28°C Degradation of phenol and pyrocatechol derivatives: 28°C
pH	p-Chloro-m-cresol degradation: Not reported Degradation of phenol and pyrocatechol derivatives: 7.3
Shaking	p-Chloro-m-cresol degradation: 220 rev./minute Degradation of phenol and pyrocatechol derivatives: none
Concentration of inoculum	p-Chloro-m-cresol degradation: Not reported Degradation of phenol and pyrocatechol derivatives: Not reported
Other relevant criteria	None

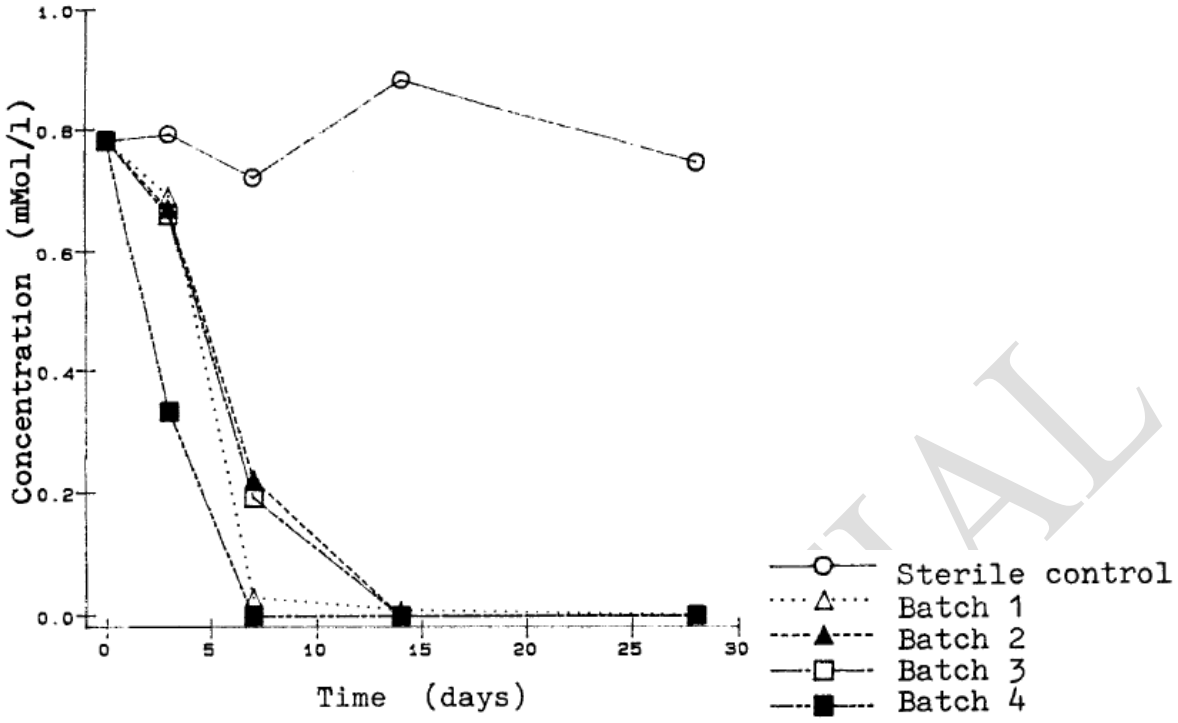

Figure A7_1_2_2_1-1: Degradation of p-chloro-m-cresol by Rhine water (initial concentration 100 mg/L)

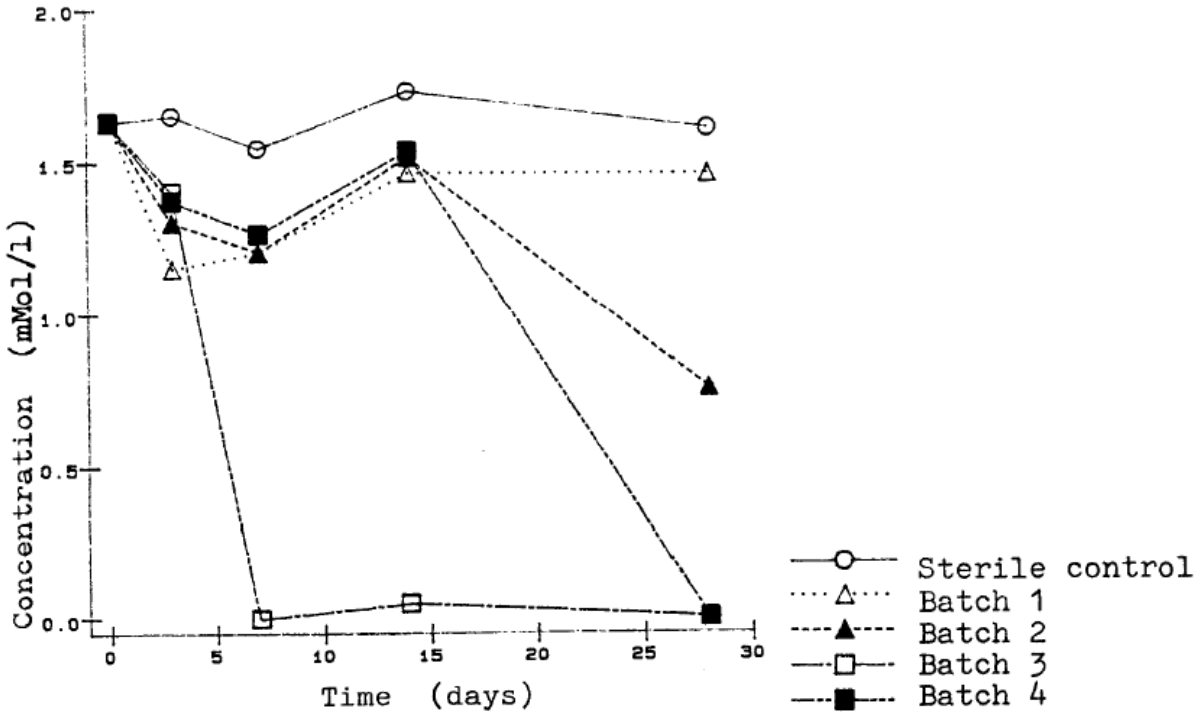

Figure A7_1_2_2_1-2: Degradation of p-chloro-m-cresol by Rhine water (initial concentration 200 mg/L)

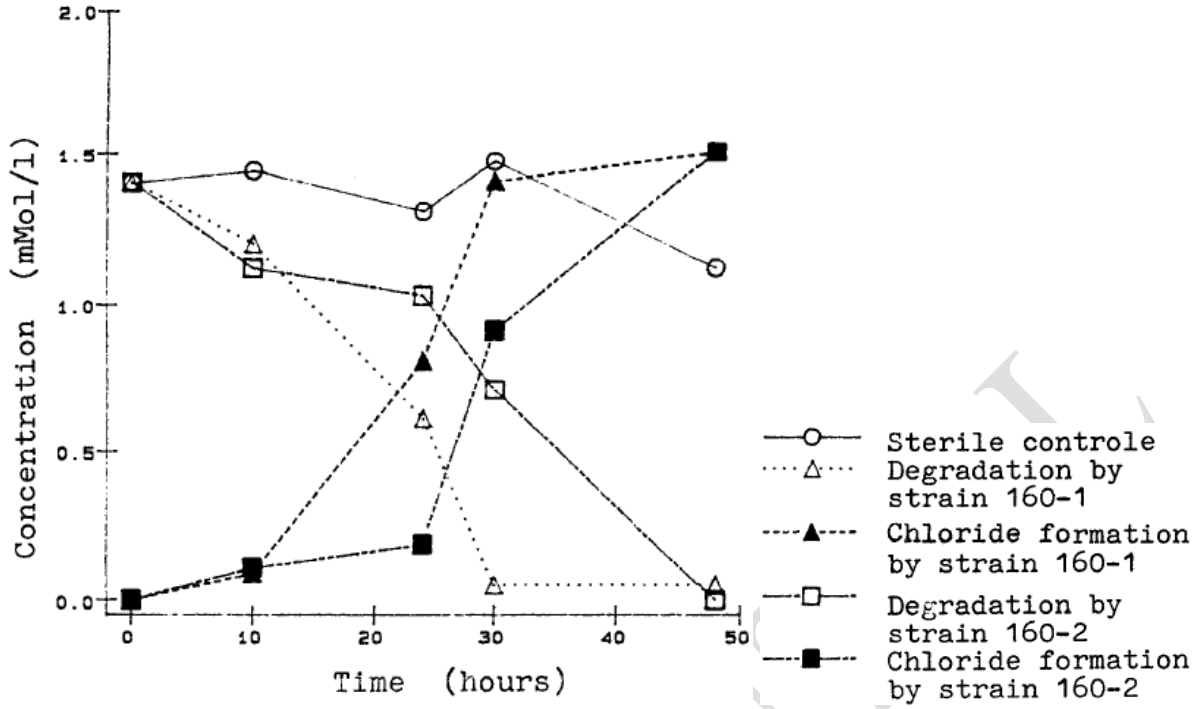

Figure A7_1_2_2_1-3: Degradation of p-chloro-m-cresol by two bacteria strains (RST 160-1 and RST 160-2) isolated from Rhine water

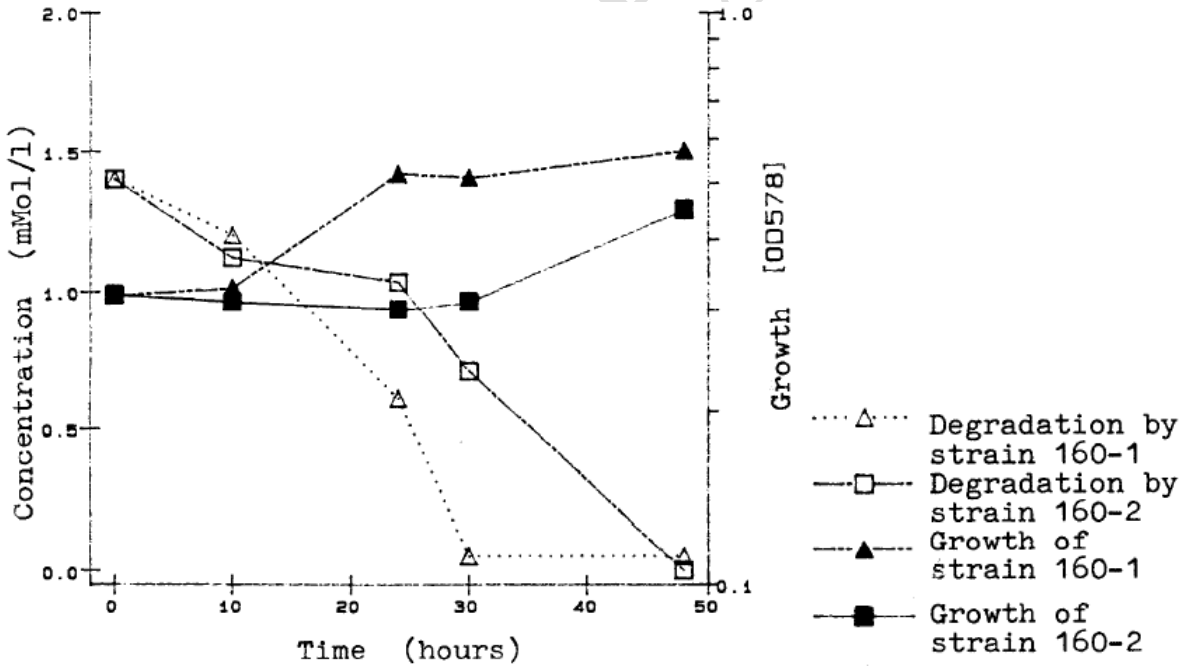

Figure A7_1_2_2_1-4: Growth with p-chloro-m-cresol of two bacteria strains (RST 160-1 and RST 160-2) isolated from Rhine water

Table A7_1_2_2_1-4: Mineralisation of various phenols by strain 160-1 after growth with p-chloro-m-cresol

Substrate	Specific O_2 uptake rate (µmol O_2/L per h and g dried solids)
Phenol	840
2-Methylphenol	940
3-Methylphenol	940
2-Chlorophenol	0
3-Chlorophenol	0
4-Chlorophenol	95
3-Methyl-4-chlorophenol	560
2-Methyl-4-chlorophenol	0
5-Methyl-4-chlorophenol	375
Pyrocatechol	4690
4-Methylpyrocatechol	19690
4-Chloropyrocatechol	16880
3,5-Dichloropyrocatechol	0
3-Methyl-4-chloropyrocatechol	4060

Section A7.1.2.2.1 Aerobic aquatic degradation study (02)

Annex Point XII 2.1

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1 REFERENCE

1.1	**Reference**	Gerharz, T. (2011): Degradation of 4-chloro-3-cresol in a liquid environment (washing water after stable cleaning – stable with laying hens) – Test Report, Lanxess Deutschland GmbH, Leverkusen, Germany. Unpublished, Date: 2011-05-26.
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; Internal test procedure developed for this experiment
2.2	**GLP**	No
2.3	**Deviations**	n.a.

3 MATERIALS AND METHODS

Section A7.1.2.2.1 Aerobic aquatic degradation study (02)

Annex Point XII 2.1

3.1	**Test material**	Washing water from a laying hen stable 4-chloro-3-cresol (Preventol® CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	-
3.1.5	TS inhibitory to microorganisms	-
3.2	**Reference substance**	No
3.2.1	Initial concentration of reference substance	-
3.3	**Test ing procedure**	
3.3.1	Test system	see Table A7_1_2_2-2
3.3.2	Test conditions	see Table A7_1_2_2-3
3.3.3	Method of preparation of test solution	After HPLC-analysis of the received washing water sample from a stable proved that no CMK residues were detectable. The CMK-free washing water was mixed and aliquoted into glass bottles at 100 g each. Afterwards one solution was spiked with 5 mg CMK/kg and the other solution with 10 mg CMK/kg by adding a one percent 4-chloro-3-cresol solution in isopropanole.
3.3.4	Initial TS concentration	5 mg CMK/ kg water and 10 mg CMK/kg water
3.3.5	Duration of test	8 days
3.3.6	Analytical parameter	Presence of CMK in washing water
3.3.7	Sampling	Sampling days: 0, 1, 3 and 8
3.3.8	Intermediates/ degradation products	Not identified
3.3.9	Controls	-
3.3.10	Statistics	The standard deviations for the arithmetic average of duplicate analytical results for each sampling event are given.

4 RESULTS

Section A7.1.2.2.1 Aerobic aquatic degradation study (02)

Annex Point XII 2.1

4.1	**Degradation of test substance**	
4.1.1	Graph	see Figures A7_1_2_2-1 and A7_1_2_2-2
4.1.2	Degradation	100 % degradation after 8 days for both concentrations
4.1.3	Other observations	-
4.1.4	Degradation of TS in abiotic control	-
4.1.5	Degradation of reference substance	-
4.1.6	Intermediates/ degradation products	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A washing water sample was received from a poultry stable, where CMK-containing disinfectant products are applied. HPLC analysis could not detect any CMK residues in the washing water. Therefore, the untreated washing water was spiked with CMK for determination of the biodegradation rate and extent of the test substance in the aqueous medium. Before the one percent CMK solution in isopropanol was added to the samples the washing water was mixed well and aliquoted to 100 g each into glass bottles. The washing water was then spiked at two concentrations: 5 mg CMK/kg and 10 mg CMK/kg water, whereas the added volume was maximal 100 µl / 100 g. Afterwards the samples were mixed well and the initial concentration of CMK was determined by HPLC analysis. For each sampling event (day 0, 1, 3 and 8) aliquots of the washing water were extracted with a one-way-pipette. The samples were centrifuged (1400 *g*, 7 min) and the supernatant was used for CMK analysis by HPLC.
5.2	**Results and discussion**	Duplicate analytical results for the aerobic aquatic degradation of CMK in washing water at two different concentrations (5 mg/kg and 10 mg/kg) show that CMK degraded with a half-life of 2-3 days. After a test period of 8 days, the 4-chloro-3-cresol concentration dropped below 1% for both test concentrations.
5.3	**Conclusion**	The test can be considered as valid for showing the fast degradation potential of CMK in washing water samples. For determination of the exact half-life of the a.s. in samples from animal stables the test can be repeated with more samples under standardized laboratory conditions.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A7.1.2.2.1 Aerobic aquatic degradation study (02)

Annex Point XII 2.1

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27/09/2011
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_2_2-1: Inoculum / Test organism

Criteria	Details
Nature	Inoculum population existing in the sample of washing water from a laying hen stable
Species	not known
Strain	not known
Source	see Sampling site
Sampling site	The sampling site is a laying hen stable from a farmer growing laying hens. The corresponding stable was treated with a CMK-containing disinfectant product before cleaning.
Laboratory culture	No
Method of cultivation	-
Preparation of inoculum for exposure	No preparation of the washing water samples was conducted before it was spiked with CMK solutions.
Pretreatment	No
Initial cell concentration	-

Table A7_1_2_2-2: Test system

Criteria	Details
Culturing apparatus	Glass bottles
Number of culture flasks/concentration	Duplicate samples of washing waters spiked with 5 mg CMK/kg and 10 mg CMK/kg water, respectively.
Aeration device	-
Measuring equipment	HPLC device
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_2_2-3: Test conditions

Criteria	Details
Composition of medium	The aqueous medium consists of a natural sample of washing water collected after cleaning of a laying hen stable. It can be assumed that the sample contains residues of a cleaning agent as well as residues of litter and faeces.
Additional substrate	No
Test temperature	Laboratory room temperature
pH	pH = 6.2 for the washing water received from a laying hen stable
Aeration of dilution water	No
Suspended solids concentration	-
Other relevant citeria	At each sampling event duplicate aliquots of the well mixed liquid were taken with a one-way pipette. Samples were centrifuged (1400 *g*, 7 min) and the supernatant was used for CMK analysis by HPLC.

Table A7_1_2_2-4: Test results for HPLC analysis on duplicate samples from washing water spiked with 5 mg CMK/kg and 10 mg CMK/kg water

Initial CMK concentration = 5 mg/kg water			
Time [days]	**Bottle 1 [mg CMK/kg water]**	**Bottle 2 [mg CMK/kg water]**	**arithmetic average ± standard deviation**
0	4.8	4.6	4.7 ± 0.14
1	5.0	4.6	4.8 ± 0.28
3	1.4	1.3	1.35 ± 0.07
8	< 0.02	< 0.02	< 0.02 ± 0.00
Initial CMK concentration = 10 mg/kg water			
Time [days]	**Bottle 1 [mg CMK/kg water]**	**Bottle 2 [mg CMK/kg water]**	**arithmetic average ± standard deviation**
0	9.3	10.0	9.7 ± 0.49
1	9.5	10.0	9.8 ± 0.35
3	3.3	2.7	3.0 ± 0.42
8	0.04	< 0.02	0.03 ± 0.01

Figure A7_1_2_2-1: Graph displaying the degradation of CMK in washing water for the initial concentration of 5 mg CMK / L water

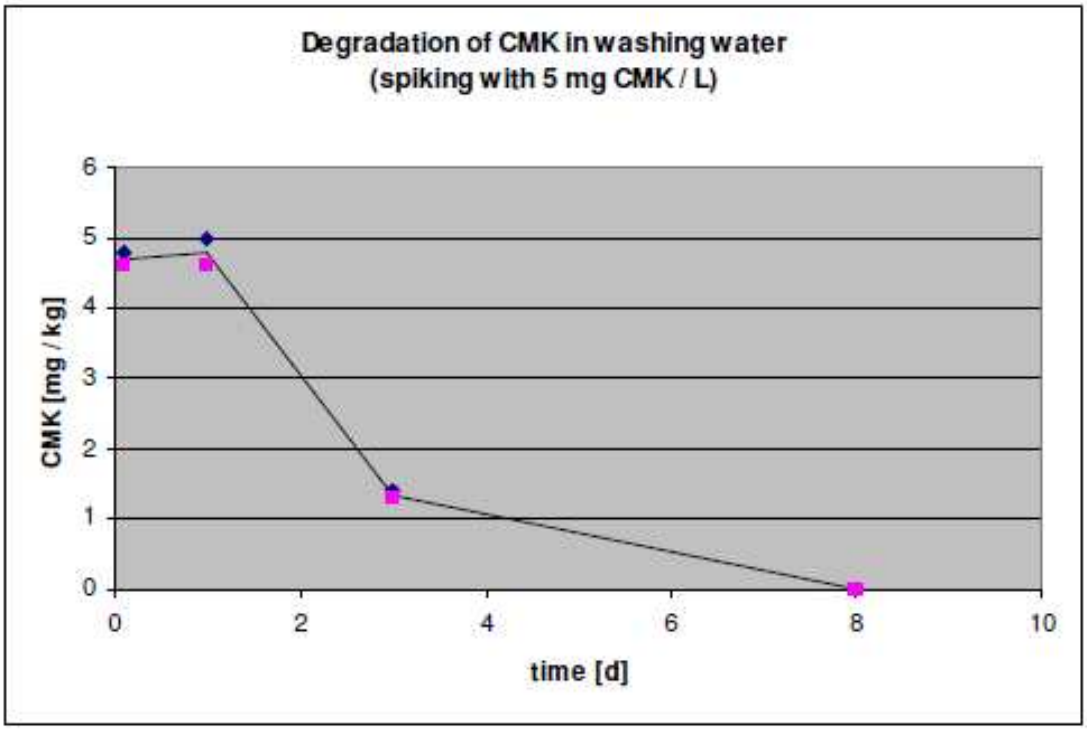
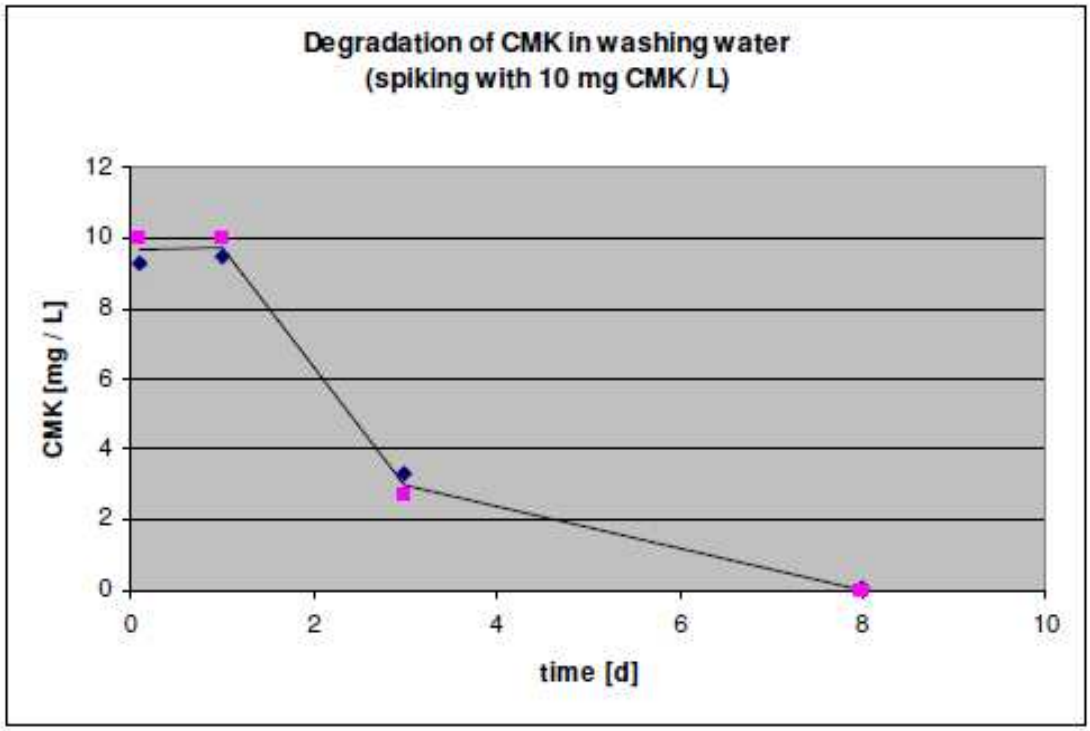


Figure A7_1_2_2-2: Graph displaying the degradation of CMK in washing water for the initial concentration of 10 mg CMK / L water



Section A7.1.2.2.1 Aerobic aquatic degradation study (01)

Annex Point IIIA, 2.2.1

Official use only

A study on the aquatic degradation of CMK is no core data requirement (*cf.* Chapter 2 of the Requirements for Biocidal Product Types: Common Core Data Set for Active Substances and Biocidal Products). In addition, the study is no requirement for the product types, for which CMK will be used (PT 1, 2, 3, 6 and 13; Chapter 2.5: Product Type specific Additional Data for Active Substances and Biocidal Products).

According to the flow scheme in Chapter 3 of the Requirements for Biocidal Product Types (Additional Data required for Active Substances and Biocidal Products) a study on the aerobic aquatic degradation is required if a biocidal substance cannot be demonstrated to be ready or inherently biodegradable and/or the substance is directly emitted to water.

For CMK, it could be proven that ready biodegradability is achievable under stringent test conditions, i.e., a high inoculum activity and/or the presence of appropriate inoculum species being able to degrade CMK. Following an adequate adaptation period of the inoculum to the compound the microorganisms are able to biodegrade CMK exhaustively, hence CMK can be considered to be inherently biodegradable (*cf.* Doc. II-A, Section 4.1.1.2).

Additionally, CMK is not direcly released to surface water bodies. As pointed out in the respective II-B Documents, the only environmental compartment for direct CMK emissions is a STP. Water bodies are – if at all - only affected indirectly, i.e., mainly via STP effluents. However, CMK present in wastewater is to a high extent biologically degradable in sewage treatment plants under aerobic conditions. The comparison of CMK in- and effluent concentrations revealed elimination rates in excess of 99% (*cf.* Doc. II-A, Section 4.1.1.2.3), indicating that a potential release to surface water bodies will be minimal, if occurring at all.

Taking into consideration the biodegradation potential of CMK and the negligible release potential of CMK to water bodies, a study on the aerobic aquatic degradation is not considered reasonable.

However, for reasons of completeness, the study of Rast & Kölbl (1987) was integrated into the dossier. The study is not designed according to a known guideline and shows deficiencies, which are already mentioned in Doc. III-A, 7.1.2.2.1. However, the study is included into the dossiers for the following reasons:

1. The test substance p-chloro-m-cresol could be shown to be degradable by microorganisms in Rhine water within a few days and without lag-phase (test concentration 100 mg/L). At higher concentrations (200 mg/L) the degradation is slower. However, these concentrations are unlikely to occur under natural conditions.

2. Isolated bacteria from the mixed cultures present in the Rhine water were able to grow using the test substance as the sole carbon source. The test substance was quantitatively degraded and dechlorinated.

The degradation of CMK in Rhine water is referred to in graphical form in the report (see Figures A7_1_2_2_1-1 and A7_1_2_2_1-1). The derivation of DT_{50} values can therefore only be done visually. For the test with 100 mg/L CMK the DT_{50} values for the four batches amount to

Section A7.1.2.2.1 Aerobic aquatic degradation study (01)

Annex Point IIIA, 2.2.1

2.5 days (one batch) and 5 to 6 days (three batches). For the test with 200 mg/L CMK the DT_{50} values amount to 5 days, 21 days and 28 days. In one of the batches at the higher test concentration 50 % degradation is not achived.

Regarding the metabolic pathway of CMK, the following results are obtained. Two gram-negative bacterial strains are isolated which are capable to mineralize CMK completely when the substance is offererd as sole carbon source. One strain showed a high phenol hydroxylase and catechol-2,3-dioxygenaseactivity which is tested with several phenolic substrates. As a metabolic intermediate of the CMK degradation, 3-methyl-4-chloropyrocatechol is postulated and shown experimentally to be degraded rapidly. An accumulation of this breakdown product in surface water is therefore not to be expected. The study by Rast & Kölbl (1987) does not allow further conclusions on the pathway of CMK degradation in water. However, during the available biodegradation tests the formation of stable metabolites or intermediates is not reported. Also monitoring data give no indication for the formation of such. Therefore, further efforts for elucidating the metabolic pathway of CMK in surface water are not deemed reasonable.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2009
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

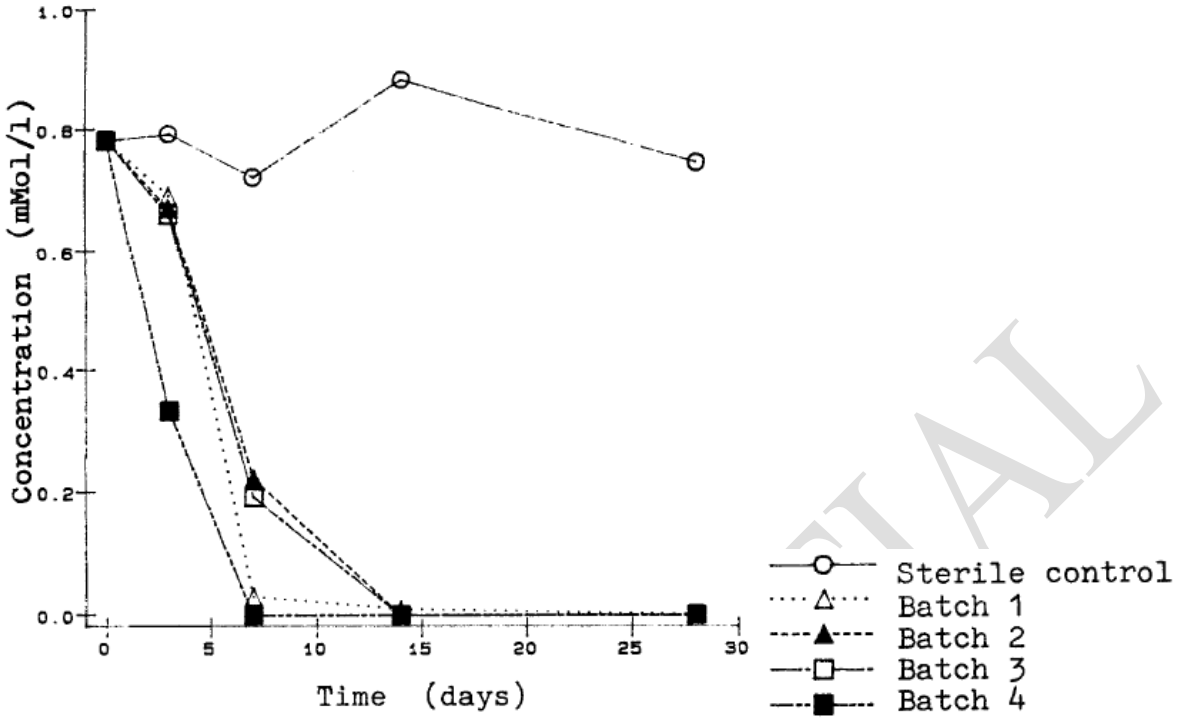


Figure A7_1_2_2_1-1: Degradation of p-chloro-m-cresol by Rhine water (initial concentration 100 mg/L)

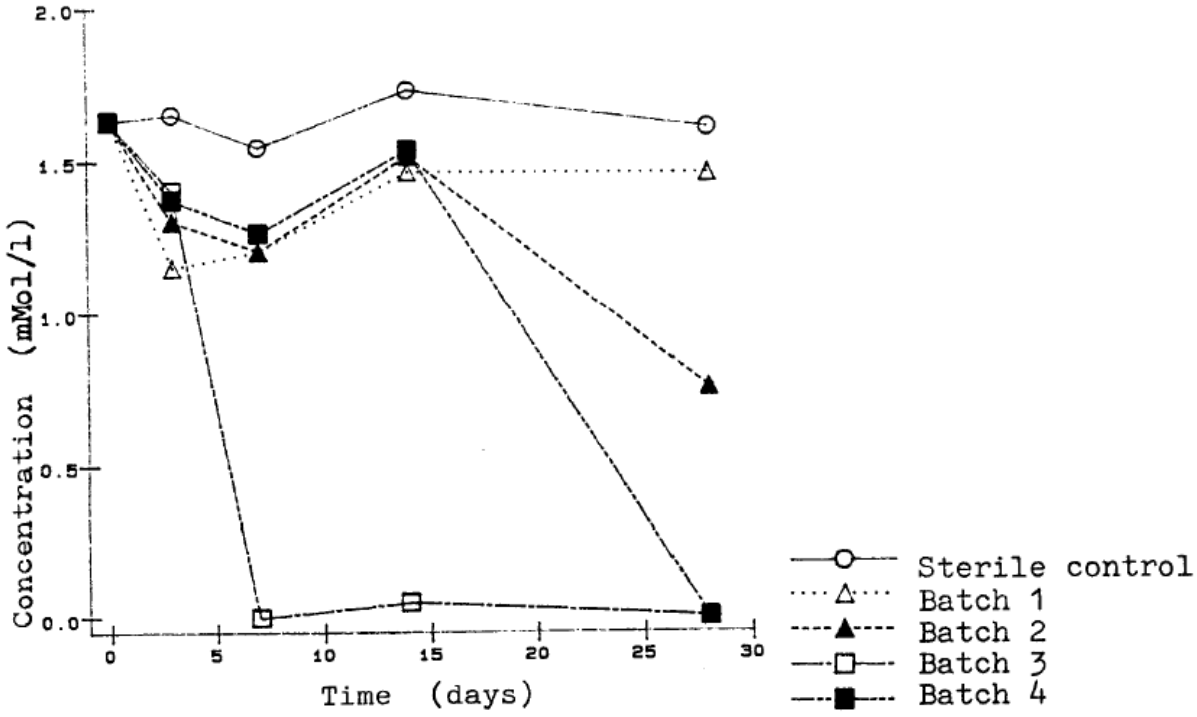


Figure A7_1_2_2_1-2: Degradation of p-chloro-m-cresol by Rhine water (initial concentration 200 mg/L)

Section A7.1.2.2.2 (01) Water/Sediment degradation study

Annex Point IIIA XII 2.1

Official use only

1 REFERENCE

1.1	**Reference**	Möndel, M. (2009): ^{14}C-Preventol CMK: Aerobic degradation of ^{14}C-Preventol CMK in two different aquatic sediment systems. RLP AgroScience GmbH, Neustadt, Germany. Study No. AS85, Date: 2009-03-26
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline for Testing of Chemicals, No. 308: „Aerobic and Anaerobic Transformation in Aquatic Sediment Systems", Date: 2002-04-24.
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	Radiolabelled 4-chloro-3-methylphenol (Synonyms: p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Refer to chapter 2 of dossier (Identity of active substance)
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Not relevant
3.1.5	Composition of Product	Test with the active ingredient, therefore not relevant
3.1.6	TS inhibitory to microorganisms	Since p-chloro-m-cresol is a microbicide, test substance concentrations were this study were chosen based on the available results of basic laboratory degradation tests.
3.1.7	Specific chemical analysis	HPLC was used for the determination of the radiochemical purity of the test item in the application solution. Separation and characterisation of the radioactivity in the water specimen and sediment extract were also done using HPLC. The test item was identified by spiking selected aliquots with the non-radiolabelled reference item of p-chloro-m-cresol.

Section A7.1.2.2.2 (01) Water/Sediment degradation study

Annex Point IIIA XII 2.1

3.2	**Reference substance**	Yes, non-radiolabelled p-chloro-m-cresol (Batch No. [redacted]) was used as chromatographic standard for radio HPLC analysis and additionally for application of the control and biomass vessels.	
3.2.1	Initial concentration of reference substance	A reference item solution (unlabelled Preventol CMK) was prepared with a nominal amount of 1 mg/mL to identify the test item during HPLC analysis. For application of the control and biomass vessels this first solution was further diluted to 200 µg/L.	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Two natural water/sediment systems ("Pond" and "River") were used in this study. The sediments of the two German aquatic test systems were characterised as a silt loam ("River" system) and sand ("Pond" location). The properties of these systems werde further described in attached Tables A7_1_2_2_2-1 and A7_1_2_2_2-2.	
3.3.2	Test system	The water/sediment system consisted of sediment and the corresponding water as a static system. All test vessels were applied individually. The actual amount of test item applied to each test vessel was determined during the application procedure. The test system is described in detail in Table A7_1_2_2_2-3.	
3.3.3	Test conditions	Aerobic conditions were established which allows some gas exchanges out of and into the water phase. The sediments remained aerobic during the test period. The test conditions are described in Table A7_1_2_2_2-4.	
3.3.4	Method of preparation of test solution	The water/sediment systems were sampled by the test facility at the described sites. The sediment was sieved through a 0.15 mm sieve. Each test system was filled with wet sediment reaching a height of 2.0-2.5 cm. Afterwards 6 cm of the corresponding water were layered over the sediment. The preparation of the test flasks is described in Table A7_1_2_2_2-3.	
3.3.5	Initial TS concentration	A concentration of 100.07 µg/test system was applied, corresponding to 200.14 µg/L test item for the water phases of both the "River" system and the "Pond" system.	
3.3.6	Duration of the test	35 days incubation period	
3.3.7	Analytical parameter	Distribution of the applied radioactivity in the different phases (water, sediment, air) was investigated. Basis analytical measurements during the test: I. Water phase: pH, redox potential, oxygen content and saturation, temperature, II. Sediment phase: pH, redox potential,	X
3.3.8	Sampling	The sampling dates for determination of the degradation of the test substance are summarised in attached Table A7_1_2_2_2-5. The analytical parameter (pH, redox potential, oxygen content and saturation, temperature) were determined either or on Monday and	

Section A7.1.2.2.2 (01) Water/Sediment degradation study

Annex Point IIIA XII 2.1

		Tuesday and either or on Thursday and Friday.	
3.3.9	Intermediates/ degradation products	The distribution of the applied radioactivity to the different phases was investigated. The amount of radioactivity in the extractable and unextractable portions of sediment was quantified.	
3.3.10	Controls	Non-radiolabelled p-chloro-m-cresol (Batch No. [redacted]) was used for application of the control and biomass vessels.	
3.3.11	Statistics	The results of the water/sediment study were evaluated using KinGUI version 1.1. For the calculation of the DT50 and DT90 values two different models were used. One overall model was used to describe the dissipation of the test substance in the whole system. A second, more detailed model (taking into account the equilibrium between the two phases) was applied to describe the dissipation in the water phases separately. The model concepts are explained in detail in chapter 2.9 of the test report.	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Degradation of TS in abiotic control	Not applicable.	
4.1.2	Degradation	<u>DT50 values:</u> The DT50 value for the entire system was calculated to be 1.22 days for the “River” system and 1.90 days for the “Pond” system, respectively. The corresponding DT50 values for the water phase were 1.07 days for the “River” system and 1.74 days for the “Pond” system, respectively. <u>DT90 values:</u> For the entire system the following DT90 values for the entire system were calculated: 4.05 days for the “River” system and 6.31 days for the “Pond” system, respectively. The corresponding DT90 values for the water phase were 3.57 days for the “River” system and 5.78 days for the “Pond” system, respectively. See also attached Table A7_1_2_2_2-6 for a summary of revealed DT50 and DT90 values.	
		Attached Table A7_1_2_2_2-7 summarises the dissipation parameters of the not identified metabolites. DT50 values for the overall system ranged between 7.0 to 37.4 days, whereas DT90 values of 23.3 and 126.0 days were calculated for “River” and “Pond” system, respectively. Due to the unsufficient separation of the metabolites in the HPLC chromatogramms the peaks overlapps and a distinct integration of each peak was not possible. Therefore, the DT50 and DT90 values for the degradation products were calculated as sum of all individual peaks.	X
		<u>Degradation products:</u> After about 14 to 28 days of incubation, the test p-chloro-m-cresol disappeared from the water phase in both aquatic systems. The amount of extractable test substance was quite similar during whole incubation period (1.6 to 2.0 % of applied radioactivity at last sampling date). After reaching maximum values 3-4 days after application, the amount of not identified metabolites decreased in both systems continuously until finalisation of the study. Accordingly, the amount of CO_2 increased and	X

Section A7.1.2.2.2 (01) Water/Sediment degradation study

Annex Point IIIA XII 2.1

		amounted to 23.9 % (“Pond”) and 37.0 % (“River”) after 35 days. The unextractable portions in the sediment residues with amounts of 46.4 % (“Pond”) and 52.4 % (“River”) seems to be stable at the end of incubation period.	X
		In the attached summary tables the distribution of radioactivity during the incubation period is given (Tables A7_1_2_2_2-8 and A7_1_2_2_2-9. Furthermore the amounts of test item and metabolites in the water/sediment systems are summarised in the Tables A7_1_2_2_2-10 and A7_1_2_2_2-11.	
4.1.3	Graph	The distribution of the radioactivity during the test period is given in graphs in the test reports (Figure 22 to 25).	
4.1.4	Other observations	Recovery: The recovery ranged from 86.2 % to 99.2 % of applied radioactivity. The mean recovery rate was 92.5 % for the water/sediment system “River” and 94.2 % of the applied radioactivity for the water/sediment system “Pond”, respectively.	X
4.1.5	Degradation of reference substance	Not applicable, since non-radiolabelled p-chloro-m-cresol (Batch No. [redacted]) was used as chromatographic standard for radio HPLC analysis and additionally for application of the control and biomass vessels..	
4.1.6	Intermediates/ degradation products	*cf.* Point 4.1.2 Up to five metabolites could be detected (HPLC) in the time range (Retention time) of 1.8 to 3.2 minutes, indicating the formation of very polar degradation products after oxidation and cleavage of the aromatic ring structure, which results in carboxyl and carbonic acid structures.	X
		Attached Figure A7_1_2_2_2-1 shows the assumed degradation sheme. These polar substances – which can be regarded as transient degradation products – are further degraded either to CO_2 or react chemically with the organic matter in the sediment. In this study these transient degradation products are mainly found in the first ten days. The resulting bound residues should afterwards be stipwise degraded to CO_2.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The purpose of the test was to determine the degradation rate of p-Chloro-m-cresol in two different aquatic model systems (“River” and “Pond”). Natural water/sediment samples were treated with the 14C-labelled test item (Preventol CMK; [redacted]p-chloro-m-cresol, radiochemical purity [redacted]) at a concentration of 100.07 µg/test system, corresponding to 200.14 µg/L test item for the water phases of both the “River” system and the “Pond” system. The test systems were incubated for a period of 35 days in the dark under aerobic conditions in the laboratory.	
		The study was performed in accordance with OECD Guideline No. 308: „Aerobic and Anaerobic Transformation in Aquatic Sediment Systems” (2002).	

Section A7.1.2.2.2 (01) Water/Sediment degradation study

Annex Point IIIA XII 2.1

5.2	**Results and discussion**	Recovery: The recovery ranged from 86.2 % to 99.2 % of applied radioactivity. The mean recovery rate was 92.5 % for the water/sediment system "River" and 94.2 % of the applied radioactivity for the water/sediment system "Pond", respectively. DT50 values: The DT50 value for the entire system was calculated to be 1.22 days for the "River" system and 1.90 days for the "Pond" system, respectively. The corresponding DT50 values for the water phase were 1.07 days for the "River" system and 1.74 days for the "Pond" system, respectively. DT90 values: For the entire system the following DT90 values for the entire system were calculated: 4.05 days for the "River" system and 6.31 days for the "Pond" system, respectively. The corresponding DT90 values for the water phase were 3.57 days for the "River" system and 5.78 days for the "Pond" system, respectively. Degradation products: After about 14 to 28 days of incubation, the test p-chloro-m-cresol disappeared from the water phase in both aquatic systems. The amount of extractable test substance was quite similar during whole incubation period (1.6 to 2.0 % of applied radioactivity at last sampling date). After reaching maximum values 3-4 days after application, the amount of not identified metabolites decreased in both systems continuously until finalisation of the study. Up to five metabolites could be detected (HPLC) in the time range (Retention time) of 1.8 to 3.2 minutes, indicating the formation of very polar degradation products after oxidation and cleavage of the aromatic ring structure, which results in carboxyl and carbonic acid structures. These polar substances – which can be regarded as transient degradation products – are further degraded either to CO_2 or react chemically with the organic matter in the sediment. Accordingly, the amount of CO_2 increased and amounted to 23.9 % ("Pond") and 37.0 % ("River") after 35 days. The unextractable portions in the sediment residues with amounts of 46.4% ("Pond") and 52.4 % ("River") seems to be stable at the end of incubation period.	X
5.3	**Conclusion**	Rapid degradation of p-chloro-m-cresol was observed in two natural water/sediment systems.	
5.3.1	Reliability	[redacted]	X
5.3.2	Deficiencies	None	X

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2009
Materials and Methods	[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Remarks	[redacted]
	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 A7_1_2_2_2-1: Inoculum / Test organisms

Criteria	Details
Nature	Two natural water/sediment systems ("Pond" and "River") were used in this study.
Species	Natural populations of microorganisms
Strain	Not relevant
Source/Sampling site	Sources of the natural water/sediment systems: System "Pond": Natural pond at Schifferstadt/Germany System "River": Rhine river, Berghausen, Germany
Laboratory culture	No (see above)
Method of cultivation	Not relevant
Preparation of inoculum for exposure	The water/sediment systems were sampled by the test facility at the described sites. The sediment was sieved through a 0.15 mm sieve. Each test system was filled with wet sediment reaching a height of 2.0-2.5 cm. Afterwards 6 cm of the corresponding water were layered over the sediment. The volume of applied water was determined by weighting to an amount of approx. 494 mL. Therefore, the water:sediment ratio in the test systems was approx. 3:1 (v:v). After preparation, the aquatic systems were equilibrated in a temperature controlled chamber under aeartion by gently stirring from the top without disturbing the sediment. Equilibration was done in the dark for about 5 to 6 weeks at 20 ± 2°C.
Pre-treatment	Upon arrival at the test facility the sediments and water phase were stored at +1 to +10°C for 2 and 3 days respectivels, until use. Thereafter the sediments were weight into the test systems and incubated for 38-39 days until treatment of the test systems with p-chloro-m-cresol.
Initial cell concentration	The microbial biomass of the sediments was determined short time before beginning of the the experiments according to the SIR (substrate-induced respiration) method.

Table A7_1_2_2_2-2: Characteristics of the water/sediment systems used (preliminary)

Number	I	II
Name	River (Berghäuser Altrhein)	Pond (Kelmetschweiher)
Origin	Rhine river at Berghausen Rhineland Palatinate Germany	Schifferstadt Rhineland Palatinate Germany
Sampling date in the field	October 28, 2008	October 28, 2008
pH at sampling site (Sediment)[2)]	7.1 (12.1°C)	6.6 (11.5°C)
pH at sampling site (Water)[2)]	7.4 (12.1°C)	7.7 (11.5°C)
RedOx-potential [mV] at sampling site (Sediment)[2)]	-190	-208
RedOx-potential [mV] at sampling site (Water)[2)]	161	141
Oxygen saturation [mg/l] at sampling site (Water)[2)]	9.36 (83.3%)	9.60 (90.0%)
DOC / TOC [mg/L][1)]	4.9	12.2
N tot. [mg/L][1)]	1.4	1.0
P tot. [mg/L][1)]	0.03	<0.02
Hardness °dH [2)]	7.6	9.2
Sediment		
Textural class (USDA)	Silt loam	Sand
Textural analysis[1)]		
< 2 µm clay [%]	7.4	1.9
50-2 µm silt [%]	75.9	1.1
2000-50 µm sand [%]	16.8	96.9
TC [%][1)]	7.30	0.30
TIC [%][1)]	2.14	0.05
Carbonates (Calculated, TIC x 5.0) [%][1)]	17.8	0.42
TOC [%][1)]	5.17	0.25
Organic Matter (Calculated, TOC x 1.72) [%][1)]	8.89	0.43
N tot. [mg/kg dry weight][1)]	3640	190
P tot.[mg/kg dry weight] [1)]	769	34.1
Cation exchange capacity pot. [1)] [mval Ba/100 g dry weight]	29.1	1.7
Maximum water holding capacity [g H_2O/ 100 g dry soil] [2)]	97.5	24.4
Microbial biomass [mg $C_{microbial}$ /kg dry soil]		
optimum amount of glucose [ppm]	6000	2000
Day 0[2)]	2.58	0.12
Day 35[2)]	6.37	0.37

[1)] Determined by Chemisches Institut Pforzheim GmbH CIP; Dr. Rainer Kiefer; March, 2009
[2)] Data determined by the Test Facility

Table A7_1_2_2_2-3: Test system

Criteria	Details
Culturing apparatus	The test was performed in air tight cylindrical 1 L metabolism flasks (inner diameter 10 cm), corresponding to a surface area of 78.5 cm². After preparation and equilibration (see Table A7_1_2_2_2-1 for details) the system was connected to a trapping system which allows trapping of volatile products (i.e. carbon dioxide) formed in the vessels.
Number of replicates/concentration	Test item: Refer to Table A7_1_2_2_2-5. Reference item / toxicity control: Two replicates. Control unit: Refer to Table A7_1_2_2_5
Measuring equipment	During equilibration, pH and oxygen content of the water and redox potential of water and sediment phase were monitored. Control vessels were additionally equipped with a platinum electrode which was completely covered with the sediment.
Oxidation reduction indicator	No

Table A7_1_2_2_2-4: Test conditions X

Criteria	**Details**
Composition of medium	Not applicable since natural water/sediment systems were used in this study.
Additional substrate	None
Solvent	The application solution contained the test substance in ACN:H_2O / 4:1 / v:v
Preparation of medium	Each test system was filled with wet sediment reaching a height of 2.0-2.5 cm. Afterwards 6 cm of the corresponding water were layered over the sediment. The volume of applied water was determined by weighting to an amount of approx. 494 mL. Therefore, the water:sediment ratio in the test systems was approx. 3:1 (v:v). After preparation, the aquatic systems were equilibrated in a temperature controlled chamber under aeartion by gently stirring from the top without disturbing the sediment.Equilibration was done in the dark for ~~about~~ 4 **5** weeks at 20 ± 2°C.
Test temperature	Incubation was done in the dark at 20 ± 2°C (recorded with data logger)
pH	pH values at sampling sites: "River"-System: 7.1 / 7.4 (sediment / water) at 12.1°C. "Pond"-System: 6.6 / 7.7 (sediment / water) at 11.5°C.
Suspended solids concentration	Not applicable for this test design.
Other relevant criteria	None

Table A7_1_2_2_2-5: Sampling dates and number of vessels that have been prepared for the study

Variant	"River"	"Pond"
Sampling Date	Water/Sediment I	Water/Sediment II
Day 0 (0.5 h)	2	2
Day 1	2	2
Day 3	2	2
Day 4	2	2
Day 7	2	2
Day 14	1	1
Day 28	2	2
Day 35	1	1
Reserve	2	2
Biomass[1)]	4	4
Controls[2)]	2	2
Sum	22	22

1) These vessels were applied with non-radiolabeled reference item and used to determine the microbial biomass at the end of the experimental phase.
2) These vessels were applied with non-radiolabeled reference item and were equipped with a platinum electrode totally covered by the sediment.

Table A7_1_2_2_2-6: Calculated Half Lives and DT90 values for the natural water/sediment systems

	"Pond" water	"Pond" sediment	"Pond" (overall system)
Kinetic model	sfo		sfo
DT50	1.74 days		1.90 days
DT90	5.78 days		6.31 days
Fit (chi2Err%)	2.17		5.67
	"River" water	"River" sediment	"River" (overall system)
Kinetic model	sfo		sfo
DT50	1.07 days		1.22 days
DT90	3.57 days		4.05 days
Fit (chi2Err%)	19.59		27.83

Table A7_1_2_2_2-7: Calculated dissipation times (Half Lives and DT90 values) of the not identified and summarisedmetabolites for the natural water/sediment systems

Not Identified Metabolites	"Pond" water	"Pond" sediment[1)]	"Pond" (overall system)
Kinetic model	sfo		sfo
DT50	36.37 days	--	37.94 days
DT90	120.83 days	--	126.02 days
Fit (chi2Err%)	5.45	--	5.72
	"River" water	"River" sediment[1)]	"River" (overall system)
Kinetic model	sfo		sfo
DT50	6.97 days	--	7.00 days
DT90	23.16 days	--	23.25 days
Fit (chi2Err%)	10.85	--	12.70

Due to the small and inhomogen extraction yield of radioactivity in the sediment extracts the dissipation times (DT50 and DT90) were not calculated.

Table A7_1_2_2_2-8: Distribution of radio activity in water sediment system "Pond"

Table 2: Distribution of radioactivity after application of [^{14}C]-Preventol CMK in the water/sediment system "Pond" during the 35 days period (individual and mean values)

(individual values)

Incubation period		Water phase	ACN/Water Extract	Reflux Extract	Quarz wool	$^{14}CO_2$	Not extractable	Mass balance
		% radioactivity applied						
0	A	96.57	1.94	0.09	n.m.	n.m.	0.12	98.72
0	B	95.96	2.14	0.08	n.m.	n.m.	0.08	98.26
1	A	72.02	2.98	0.35	0.02	0.19	23.66	99.22
1	B	72.47	3.60	0.41	0.01	0.15	21.59	98.23
3	A	48.93	4.89	0.43	0.02	0.86	37.74	92.87
3	B	48.47	5.12	0.58	0.01	1.00	38.41	93.58
4	A	46.83	5.19	0.46	0.01	1.73	38.40	92.62
4	B	43.90	4.11	0.37	0.01	2.76	41.78	92.93
7	A	35.16	5.74	0.43	0.01	4.48	45.70	91.52
7	B	32.77	4.96	0.40	0.01	4.15	48.82	91.11
14	A	25.72	5.25	0.48	0.01	9.93	52.16	93.55
28	A	16.33	4.32	0.46	0.04	17.15	54.03	92.33
28	B	14.93	4.55	0.45	0.02	17.49	54.36	91.80
35	A	17.78	3.79	0.39	0.01	23.92	46.40	92.29

n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured

(mean values)

Incubation period	Water phase	ACN/Water Extract	Reflux Extract	Quarz wool	$^{14}CO_2$	Not extractable	Mass balance
	% radioactivity applied						
0	96.27	2.04	0.05	n.m.	n.m.	0.10	98.49
1	72.25	3.29	0.38	0.02	0.17	22.63	98.73
3	48.70	5.01	0.51	0.02	0.93	38.08	93.23
4	45.37	4.65	0.42	0.01	2.25	40.09	92.78
7	33.97	5.35	0.42	0.01	4.32	47.26	91.32
14	25.72	5.25	0.48	0.01	9.93	52.16	93.55
28	15.63	4.44	0.46	0.03	17.32	54.20	92.07
35	17.78	3.79	0.39	0.01	23.92	46.40	92.29

n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured

Table A7_1_2_2_2-9: Distribution of radio activity in water sediment system "River"

Table 3: Distribution of radioactivity after application of [^{14}C]-Preventol CMK in the water/sediment system "River" during the 35 days period (individual and mean values)

(individual values)

Incubation period		Water phase	ACN/Water Extract	Reflux Extract	Quarz wool	$^{14}CO_2$	Not extractable	Mass balance
		% radioactivity applied						
0	A	96.40	1.48	0.07	n.m.	n.m.	0.11	98.06
0	B	96.85	1.67	0.04	n.m.	n.m.	0.10	98.66
1	A	68.35	10.59	0.78	0.02	0.22	15.85	95.81
1	B	68.51	10.71	0.55	0.01	0.18	15.21	95.17
3	A	35.21	6.18	0.89	0.01	2.12	45.17	89.58
3	B	37.71	4.33	0.57	n.d.	3.35	40.27	86.23
4	A	31.40	4.81	0.44	n.d.	3.44	46.66	86.75
4	B	31.55	4.79	0.54	0.01	3.78	48.13	88.80
7	A	22.63	12.18	0.76	0.01	6.34	50.07	91.99
7	B	26.03	17.24	0.92	0.01	5.78	42.80	92.78
14	A	15.28	5.83	0.57	0.01	16.19	54.34	92.22
28	A	2.70	2.72	0.38	0.01	36.64	50.13	92.58
28	B	2.45	2.53	0.34	n.d.	41.28	45.33	91.93
35	A	2.43	2.77	0.39	n.d.	36.99	52.43	95.01

n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured

(mean values)

Incubation period	Water phase	ACN/Water Extract	Reflux Extract	Quarz wool	$^{14}CO_2$	Not extractable	Mass balance
	% radioactivity applied						
0	96.63	1.58	0.06	n.m.	n.m.	0.11	98.36
1	68.43	10.65	0.67	0.02	0.20	15.53	95.49
3	36.46	5.26	0.73	0.01	2.74	42.72	87.91
4	31.48	4.80	0.49	0.01	3.61	47.40	87.78
7	24.33	14.71	0.84	0.01	6.06	46.44	92.39
14	15.28	5.83	0.57	0.01	16.19	54.34	92.22
28	2.58	2.63	0.36	0.01	38.96	47.73	92.26
35	2.43	2.77	0.39	n.d.	36.99	52.43	95.01

n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured

Table A7_1_2_2_2-10: Amount of test item and metabolites in water sediment system "Pond"

Table 4: Amount of test item and metabolites in the water/sediment system „Pond" during the incubation period in the water phase, extract, and overall

(individual values)

Incubation Period		Preventol CMK			Not Identified Radioactivity		
		Water Phase	Extract	Overall	Water Phase	Extract	Overall
		% radioactivity applied					
0	A	96.57	1.94	98.51	0.00	0.00	0.00
0	B	94.57	2.14	96.71	1.39	0.00	1.39
1	A	62.94	0.66	63.60	9.08	2.32	11.40
1	B	67.48	1.32	68.80	4.99	2.28	7.27
3	A	29.47	3.55	33.02	19.46	1.33	20.79
3	B	28.00	4.08	32.08	20.47	1.04	21.51
4	A	26.55	3.87	30.42	20.28	1.32	21.60
4	B	10.23	1.69	11.92	33.67	2.42	36.09
7	A	8.66	3.50	12.16	26.50	2.24	28.74
7	B	5.77	3.07	8.84	27.01	1.89	28.90
14	A	1.72	3.34	5.06	24.00	1.91	25.91
28	A	0.00	3.44	3.44	16.33	0.89	17.22
28	B	0.00	2.71	2.71	14.93	1.83	16.76
35	A	0.00	1.95	1.95	17.78	1.84	19.62

n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured
results used for DT50/DT90 modelling (KinGUI 1.1)

(mean values)

Incubation Period	Preventol CMK			Not Identified Radioactivity		
	Water Phase	Extract	Overall	Water Phase	Extract	Overall
	% radioactivity applied					
0	95.57	2.04	97.61	0.70	0.00	0.70
1	65.21	0.99	66.20	7.035	2.30	9.335
3	28.74	3.82	32.55	19.97	1.19	21.15
4	18.39	2.78	21.17	26.98	1.87	28.85
7	7.22	3.29	10.50	26.76	2.07	28.82
14	1.72	3.34	5.06	24.00	1.91	25.91
28	0.00	3.08	3.08	15.63	1.36	16.99
35	0.00	1.95	1.95	17.78	1.84	19.62

n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured

Table A7_1_2_2_2-11: Amount of test item and metabolites in water sediment system "River"

Table 5: Amount of test item and metabolites in the water/sediment system „River" during the incubation period in the water phase, extract, and overall

(individual values)

Incubation Period		Preventol CMK			Not Identified Radioactivity		
		Water Phase	Extract	Overall	Water Phase	Extract	Overall
		% radioactivity applied					
0	A	95.12	1.48	96.60	1.28	0.00	1.28
0	B	95.91	1.67	97.58	0.94	0.00	0.94
1	A	64.81	9.61	74.42	3.54	0.98	4.52
1	B	64.40	9.82	74.22	4.11	0.88	4.99
3	A	7.43	0.54	7.97	27.78	5.64	33.42
3	B	0.00	4.33	4.33	37.71	0.00	37.71
4	A	2.59	4.81	7.40	28.81	0.00	28.81
4	B	0.90	2.72	3.62	30.65	2.08	32.73
7	A	1.70	12.18	13.88	20.93	0.00	20.93
7	B	11.49	17.24	28.73	14.54	0.00	14.54
14	A	0.00	4.87	4.87	15.28	0.96	16.24
28	A	0.00	1.49	1.49	2.70	1.23	3.93
28	B	0.00	1.31	1.31	2.45	1.22	3.67
35	A	0.00	1.43	1.43	2.43	1.34	3.77

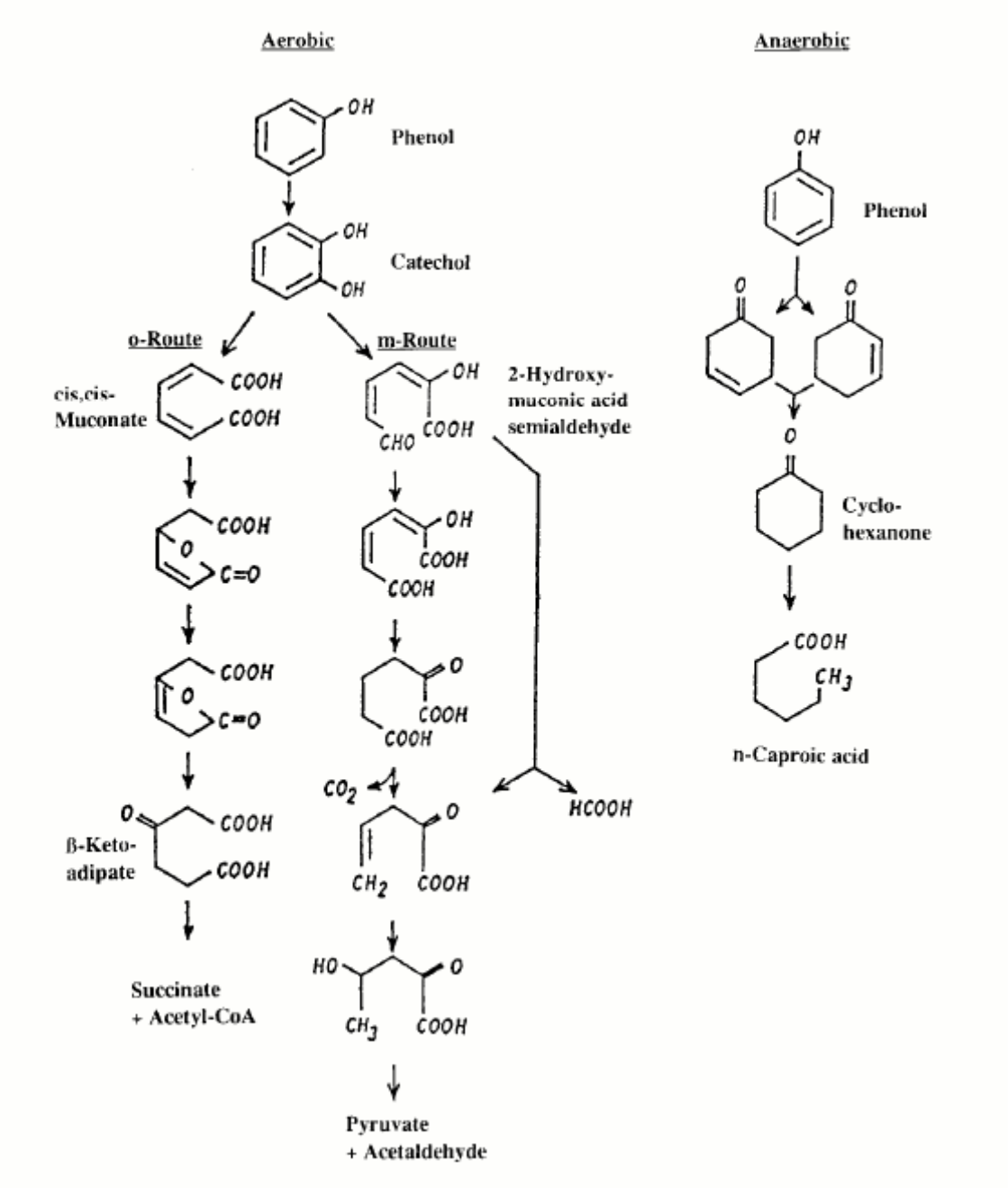
n.d. = not detected (<0.01% radioactivity applied)
n.m. = not measured
results used for DT50/DT90 modelling (KinGUI 1.1)

(mean values)

Incubation Period	Preventol CMK			Not Identified Radioactivity		
	Water Phase	Extract	Overall	Water Phase	Extract	Overall
	% radioactivity applied					
0	95.52	1.58	97.09	1.11	0.00	1.11
1	64.61	9.72	74.32	3.825	0.93	4.755
3	3.72	2.44	6.15	32.75	2.82	35.57
4	1.75	3.77	5.51	29.73	1.04	30.77
7	6.60	14.71	21.31	17.74	0.00	17.74
14	0.00	4.87	4.87	15.28	0.96	16.24
28	0.00	1.40	1.40	2.58	1.23	3.80
35	0.00	1.43	1.43	2.43	1.34	3.77

n.m. = not measured

Figure A7_1_2_2_2-1: Mechanism of the biodegradation of phenols in water, sediment and soil



Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

			Official use only
		1 REFERENCE	
1.1	**Reference**	Möndel, M. (2010): ^{14}C-Preventol CMK: Characterisation of non-identified radioactivity of ^{14}C-Preventol CMK in an aquatic sediment system. RLP AgroScience GmbH, 67435 Neustadt, Germany. Report No. AS139, unpublished, Date: 2010-05-21.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	In parts according to OECD Guideline for Testing of Chemicals, No 308 "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems", April 24, 2002.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, due to the necessity for tracking the transient metabolites of Preventol CMK and their environmental fate an appropriate chromatographic method need to be developed. The purpose of this method is to identify and quantify the metabolites clearly after maximum amounts of non-identified radioactivity (NIR) are measured.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-labelled Preventol CMK: 4-Chloro-3-methyl[U-^{14}C]phenol	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	4-Chloro-3-methyl[U-^{14}C]phenol: 592 MBq/mmol (4.14 MBq/mg)	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Molecular weight: 142.6 g/mol	
3.1.5	Method of analysis	<u>General approach:</u> After an equilibration period of 48 days the two incubation vessels with the water/sediment systems were treated with the radioactive labelled CMK solution. The test vessels were incubated at 20 ± 2 °C in the dark and water samples were analysed daily until the maximum amount of NIR was detected. Subsequently, the entire water phase was collected and stored at -18°C in the dark. This water containing the maximum amount of non-identified degradation products was then submitted to further analytical steps.	

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

During the equilibration and incubation phase the following water and sediment parameters were determined:

1. Oxygen content and saturation was measured for the water phase;
2. pH of the water phase;
3. pH of the sediment;
4. Redox potential for the water phase;
5. Redox potential for the sediment phase by using the platinum electrode of the incubation vessel:
6. Temperature of the water phase.

Measurement of Radioactivity (RA):

Aliquots of 0.5 mL - 1.5 mL of the water phase for LSC- and HPLC-analysis were taken 30 min after application and then daily until the maximum peak of the NIR seemed to be reached at day 7.

RA in the liquid samples was determined on a Canberra Packard liquid scintillation counter (TRI-CARB 2550 TR/LL or TRI-CARB 2300 TR) using Ultima Gold™ scintillator or Roth Eco Plus.

Specimen counting time was usually 10 minutes unless a 2s-criteria of less than 0.5% was reached. For each LSC-analysis the background was determined using a blank specimen. The background was automatically substracted from the LSC-values. A quench and counting efficiency correction for transformation of gross counts (cpm) into dpm/Bq was automatically performed by the instrument.

High Performance Liquid Chromatographic (HPLC) characterisation

The radiolabeled material was analysed by High Pressure Liquid Chromatography (HPLC) using radioflow detection. The determination of the radiochemical purity of the test item and the determination of the maximum amount of NIR in the total water phase was determined (in conjunction with LSC measurements) using the already executed HPLC method for the retention of the test item from a former study[1] on Preventol CMK. This method is named in the following HPLC-method 1. A second method executed in this study is HPLC-method 2, which was introduced to realise a good chromatographic separation of the formatted NIR.

For the analytic process this means that HPLC-method 1 was implemented to separate the non-identified metabolites from the test item by cutting out the eluate at the corresponding time line (1.0 to 4.5 minutes). Afterwards the fractionated eluates were combined and concentrated, followed by chromatographic analysis conducted by HPLC-method 2.

[1] Moendel, M. (2009): Aerobic degradation of ^{14}C-Preventol CMK in two different aquatic sediment systems, Study No. AS85, April 15, 2009.

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

The analytical conditions for the HPLC-method 1 were as follows:

Column:	LiChrospher 100 RP8; 250 mm x 4.00 mm, 5 µm particle size
Pre-column:	LiChrospher 100 RP8; 4.00 mm x 4.00 mm, t µm
Column Oven:	40°C
Pump:	Jasco Gradient Pump PU-1580 (Jasco Deutschland GmbH)
Mixer:	Jasco LG 980-02 S (Jasco Deutschland GmbH)
Auto Injector:	Jasco Autosampler AS-1555 (Jasco Deutschland GmbH)
Mobile Phase:	Eluent A: Acetonitrile, Eluent B: Water
Flow rate:	1.5 mL/min
Wavelangth:	215 nm
Gradient:	Eluent A: 10% in 0 min, 90% in 20 and 25 min., 10% in 26 and 30 min Eluent B: 90% in 0 min, 10 % in 20 and 25 min, 90% in 26 and 30 min
UV-Detector:	Jasco UV 1575 (Jasco Deutschland GmbH)
^{14}C-Detector:	Radio-HPLC-Detector 5000 TR series (Canberra Corp.), equipped with 500 µL liquid scintillation cell
Backgroud substraction:	13.8 cpm

The analytical conditions for the HPLC-method 2 were as follows:

Column:	ProntoSIL SC-04 EnviroPHE 7.0 µm, KNAUER; 125 mm x 4.0 mm
Pre-column:	LiChrospher C18; 4.0 mm x 3.0 mm
Column Oven:	20°C
Pump:	Jasco Gradient Pump PU-1580 (Jasco Deutschland GmbH)
Mixer:	Jasco LG 980-02 S (Jasco Deutschland GmbH)
Auto Injector:	Jasco Autosampler AS-1555 (Jasco Deutschland GmbH)
Mobile Phase:	Eluent A: Water / 1% HAc, Eluent B: MeOH / 1% HAc
Flow rate:	1.0 mL/min
Wavelangth:	280 nm
Gradient:	Eluent A: 95% in 0 min, 0% in 30, 95% in 35 and 40 min Eluent B: 5% in 0 min, 100 % in 30 min, 5% in 35 and 40 min
UV-Detector:	Jasco UV 1575 (Jasco Deutschland GmbH)
^{14}C-Detector:	Radio-HPLC-Detector 5000 TR series (Canberra Corp.), equipped with 500 µL liquid scintillation cell
Backgroud substraction:	13.8 cpm

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

Limit of Detection (LOD)

For determination of the detection limit three radio-HPLC runs without injection of radioactive compounds were conducted. The entire radioactive signal of the runs was marked (signal unit: cpm). The calculation of the background was performed by generating the cpm-mean value, e.g. 6.9 cpm, of all three HPLC-runs. For all following HPLC-runs the two fold of the determined background (e.g. 13.8 cpm) was subtracted from each run. Approximately the three fold of the background was defined as LOD, e.g. 50 cpm. The value was obtained setting a background of 13.8 cpm on HPLC and about three times this value for the detection of a peak. All signals higher than 50 cpm and with a minimum area of 100 area units were integrated.

Non-radiolabeled stock solutions with concentrations of 1 mg/L were prepared of all reference items and used for co-chromatography. The detection was performed by UV.

3.2 Reference substance

- non labelled Preventol CMK: 4-Chloro-3-methylphenol
  Batch: [redacted],
  molecular weight: 142.6 g/mol,
  molecular formula: C_7H_7ClO
- Phenol: Batch: [redacted],
  molecular weight: 94.11g/mol,
  assay: [redacted]
- 2-Nitrophenol: Batch: [redacted],
  molecular weight: 139.11g/mol,
  assay: [redacted]
- 2,4,6-Trichlorophenol: Batch: [redacted],
  molecular weight: 197.45 g/mol,
  assay: [redacted])

3.2.1 Method of analysis for reference substance: HPLC (see point 3.1.5)

3.3 Water/sediment systems

A natural water/sediment system was taken from the Kellmetschweiher located near Böhl-Iggelheim (Rhineland-Palatinate/ Germany) on October 13, 2009. The water was sampled at a depth of 30 cm and the sediment from the top 15 cm of the system.

Fundamental parameters of water and sediment were determined before sampling took place. A further characterization of both phases was conducted by Chemisches Institut Pforzheim GmbH.

According to USDA nomenclature the sediment was classified as sand. The water-sediment characteristics are shown in Table A7_1_2_2_2-1.

3.4 Testing procedure

3.4.1 Test system

Each of the two test systems, consisting of sediment and water as a static system, was filled with wet sediment to a height of about 2-2.5 cm (corresponding to approximately 305 g dry weight) and with about 500 mL water to achieve a water column of about 6 cm. Thus, the water:sediment ratio in the test systems was approx. 3:1 (v:v). The study

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

		was performed in air tight cylindrical 1 L metabolism flasks (i.d. 10 cm), corresponding to a surface area of 78.5 cm².
		After preparation, the aquatic systems were pre-equilibrated in a temperature controlled chamber under continuous aeration by gentle stirring from the top without disturbing the sediment. During equilibration, pH and oxygen content of the water and redox potential of water and sediment were monitored.
		Each system was connected to a trapping system consisting of a glass tube filled with soda lime (adsorbent for atmospheric $^{14}CO_2$) and oil wetted quartz wool (adsorbent for volatile organic compounds). Aerobic conditions were assured by gas exchange through the trapping system. Before sampling of the test vessels the air inside the system was purged through the trapping system for several minutes to ensure that possibly formed volatile radioactivity is caught before study start.
		Control vessels were additionally equipped with a platinum electrode which was completely covered with the sediment.
		The flasks were then incubated under aerobic conditions at 20 ± 2°C in the dark. During the incubation period oxygen concentration and temperature was measured for the water phase, whereas pH and redox potential was recorded for the water and sediment phase at appropriate intervals.
		^{14}C-Preventol CMK was applied in MeOH at concentrations of 0.76 mg/L and 0.78 mg/L (LSC measurement) in the two test vessels, respectively. The radiochemical purity of the test item after application was determined to be [redacted].
3.4.2	Test solutions	The two application solutions were prepared by dissolving 0.38 mg and 0.39 mg labelled ^{14}C-Preventol CMK in 2.5 mL MeOH. Thus the nominal concentrations of the application solutions were 0.76 mg/L and 0.78 mg/L, respectively.
		The content of radioactivity in the application solution was determined by LSC.

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

3.4.3	Sample preparation	Preparation of the water-sediment samples for the study was achieved by sieving the sediment through a 2.0 mm sieve and filtering the water through a 0.15 mm sieve at the test facility. Thereafter the sediment was weighed into the test systems and pre-equilibrated for 48 days until treatment of the test system with Preventol CMK. Pre-equilibration was done in the dark at 20°C ± 2°C from October, 20, 2009 until December 07, 2009. Two water/sediment test systems were treated with 0.38 mg and 0.39 mg radiolabeled Preventol CMK, respectively. This corresponded to 0.76 and 0.78 mg a.i./L considering a water content of about 500 mL in each test vessel. The application solutions were applied directly to the water surface of the test systems. During the application procedure the pipette was gently moved in order to obtain a homogenous dissipation of the test item in the water phase of the incubation vessels. Afterwards the trapping systems were connected and the stirrers were started. The radiochemical purity of the test item after application was measured to be [redacted]. Stock solutions of all four reference items were prepared with concentrations of 1mg/mL and used for chromatographic purposes.
3.4.4	Test concentration	The two test concentrations of ^{14}C-labeled Preventol CMK application solutions were 0.76 mg/L and 0.78 mg/L (LSC measurement), or 0.38 mg and 0.39 mg Preventol per 500 mL water, respectively. The test item was applied in 2.5 mL MeOH for both test solutions before each solution was added to the water phase of the corresponding test vessel.
3.4.5	Test conditions	Incubation: aerobic (water phase), essentially anaerobic (sediment) Light: dark Temperature: 20 ± 2 °C Volatiles: Carbon dioxide was trapped in glas tubes filled with soda lime. Adsorption of volatile organic degradation products was guaranteed by paraffin oil wetted glass wool, which covered the soda lime.
3.4.6	Duration of the test	Up to 7 days
3.4.7	Number of replicates	Two replicate test systems
3.4.8	Sampling	Since most of the NIR in the former study was determined in the water phase, only this phase was sampled. Sampling intervals: 0.5 hours (30 min after application), 1, 2, 3, 4, 5, 6 and 7 days

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

3.4.9 Preparation of test samples for analysis

Preparation of the water phase for parameter characterisation:

At each sampling date aliquots of the water phase for LSC- and HPLC-analysis were taken until the maximum peak of the NIR seemed to be reached at day 7 (maximum on day 6). Thereafter, the incubation was stopped and the remaining water phase was collected and split in small portions into several storage vessels to ensure safe storage conditions. Subsequently the vessels were stored at temperature conditions ≤ -18°C.

Preparation of test samples for analysis:

For the characterization of the NIR in the water phase it was necessary to concentrate the radioactivity. In order to avoid (radioactive) sampling losses due to the evaporation process (volatilisation of remaining unchanged test items could occur) the NIR was separated from the test item before evaporation using HPLC-method 1. Therefore, the HPLC-eluate within the time range of 1.0 to 4.5 minutes was cut-out by tenfold repetition of this step. Afterwards these eluates were combined and they were concentrated by rotary evaporation. In a second step the NIR contained in the concentrated samples were analysed using HPLC-method 2.

Co-chromatography was carried out with selected samples, which were spiked with the non-radioactive reference item phenol.

4 RESULTS

4.1 Water/sediment distribution and metabolism

The chromatographic runs of the HPLC analysis of ^{14}C-Preventol CMK in water/sediment-system revealed seven base line separated peaks, which are presented in Table A7_1_2_2_2-2 and expressed in percent of the applied radioactivity.

In Table A7_1_2_2_2-3 the relationship between the total amount of NIR on day 7 (22.8%) and the results of HPLC-analysis (total peak area = 100%) is presented. The separation and quantification of the peaks achieved by the new developed HPLC-method 2 shows that no metabolite exceeded more than 6.9% AR (n.i.-4) after reaching the maximum amount of NIR seven days after incubation.

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

4.2	**Specification of transformation products**	For quantification and specification of the seven base line separated Peaks see Table A7_1_2_2_1-3. The chromatogram of the separated and concentrated water phase on day 7 of the study is presented in Figure A7_1_2_2_2-1. The corresponding Peak Area Report is presented in Table A7_1_2_2_2-5. After separation and quantification of the non-identified metabolites and determination of their corresponding retention time, selected samples were spiked with the reference item phenol for co-chromatography. Identical retention times were determined for peak n.i.-4 and phenol (*cf.* Figure A7_1_2_2_2-2 & Table A7_1_2_2_2-5 for the Peak Area Report). Short retention times of n.i.-1 and n.i.-2 are indicating the formation of very polar degradation products after oxidation and/or cleavage of the aromatic ring structure. The longer retention times of the remaining degradation products (n.i.-5 to n.i.-7) are indicating the perpetuation of the aromatic ring structure. A hydroxylation of the methyl group and then in the further process a oxygenation to aldehydic and carboxylic structure is assumed.	
4.3	**Calculations**	The HPLC results of the water samples of this study (day 7) provide the basis for a recalculation of the results of the former study. Therefore, the sample holding the maximum amount of NIR measured in the former study (Day 3, River system) and the sample containing the maximum amount of NIR measured on the Day 7 (Pond system) were used. The assigned amounts of RA of the individual peaks ranged between 2.4% AR and 9.9% AR (mean values) in the water phase of day three in the former study. Due to the fast degradation behaviour of the metabolites the amount of NIR decreased in the water samples of day 7 and ranged between 1.0% AR and 8.1% AR (former study). It can be assumed that none of the individual metabolites of the former study exceeded 9.9% AR corresponding to 19.8 µg/L CMK equivalents. For details of the reported values refer to Table A7_1_2_2_2-4.	X
4.4	**Dissipation time**	The half-lifes (DT_{50} values) of the seven formed degradation products of ^{14}C-Preventol CMK were very short and ranged between 7.0 and 36 days.	X

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

Annex Point IIIA, XII.2.1.

5.1	**Materials and methods**	The objective of the study was to provide information on the numbers and order of magnitude of the formed non identified metabolites after development of an appropriate chromatographic method. To achieve this study design was adopted from the already conducted study on the aerobic degradation of ^{14}C-Preventol CMK by Möndel (2009).	
		A water-sediment test system was sampled from Kellmetschweiher near Böhl-Iggelheim (Rhineland-Palatinate/ Germany).	X
		The study was performed in air tight cylindrical 1 L metabolism flasks. The water was filtered through a 0.15 mm sieve and the sediment was sieved through a 2.0 mm screen.	
		Two incubation vessels were incubated at 20 ± 2°C in the dark.	
		The water/sediment test systems were treated with 380 µg and 390 µg of radiolabelled Preventol CMK per 500 mL water per test vessel. The radiochemical purity of the test item after application was measured to be [redacted].	
		The test item was applicated at actual concentrations of 0.76 and 0.78 mg a.i./L water, respectively.	
		After reaching maximum amounts of non-identified degradation products of Preventol CMK the radioactivity in the water phase was worked up and analysed by LSC and HPLC. For HPLC analysis two different methods were applied to allow for the determination of the distinctive radioactive peaks and their respective quantification.	
5.2	**Results and discussion**		
5.2.1	Distribution and characterisation of the degradation products	Two water/sediment test systems were treated with 0.38 mg and 0.39 mg radiolabelled Preventol CMK, respectively. One replicate of the incubation systems showed a maximum formation of NIR six days after treatment and amounted to 23.9% AR. Therefore, the test was stopped seven days after application and the water phase was collected and used for further characterisation of NIR applying a specially developed HPLC-method. On day 7 after treatment the water phase sample contained 22.8% of AR.	
		A similar order of magnitude for the analysed AR (26.8%) was detected in the first study on the degradation of Preventol CMK in aquatic water/sediment systems.	X
		The HPLC analysis of the NIR was carried out after separation and concentration of NIR and showed up to seven base line separated peaks (Figure A7_1_2_2_2-1.). The retention times of the seven distinct metabolites range between 1.1 and 18.9 minutes for the first HPLC run, and for the second HPLC run they range between 1.27 and 18.80 minutes (*cf.* Table A7_1_2_2_2-5). Two of them range in the category of short retention times (up to only 2.5 and 2.47 minutes, respectively), one metabolite proves to have a retention time of about 4.5 - 4.7 minutes and the remaining four substances reach values between 10.0 up to 18.9 minutes and 10.20 up to 18.80 minutes, respectively. The corresponding mean values for the total peak area start at 6.4% AR (n.i.-3) and go up to a maximum value of 30.3% AR (n.i.-4). For details on the retention times and percentage peak area distribution refer to Table A7_1_2_2_2-2.	

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

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		By carrying out co-chromatography with selected samples, which were spiked with the reference item phenol, identical retention times were determined for peak n.i.-4 and phenol (*cf.* Figure A7_1_2_2_2-2 and Table A7_1_2_2_2-6). Short retention times of n.i.-1 and n.i.-2 indicate the formation of very polar degradation products after oxidation and/or cleavage of the aromatic ring structure. The longer retention times of the remaining degradation products allow the assumption that the aromatic ring structure is perpetuated. A hydroxylation of the methyl group and then in the further process an oxygenation to aldehydic and carboxylic structures is assumed. HPLC results gained due to the new developed chromatographic method proved that the former non-separated and non-identified peaks could be split by the new analysis. Furthermore the method showed that no metabolite exceeded more than 6.9% AR (n.i.-4) after reaching the maximum amount of NIR seven days after incubation.
5.2.2	Correlation of study results	Due to the insufficient separation of detected metabolites of Preventol CMK in a former water/sediment study, this second study was initiated to clarify the numbers and order of magnitude of degradation products by developing an appropriate chromatographic method. To achieve this purpose the same study design as in the first study was applied with the exception that the test systems were treated with the four-fold amount of radioactive labeled Preventol CMK. The test was stopped seven days after application of the test item and one day after formation of the maximum amount of non-identified metabolites. On the last day of incubation the amount of NIR was determined to be 22.8% AR. The water phase was gradually used for method development and subsequently for analysis. The successful appliance of the new developed HPLC method revealed up to seven baseline separated metabolites. Quantification of their corresponding peak areas showed that none of them exceeded 6.9% AR. The detailed outcome of this study was then used to re-evaluate the results of the first water/sediment study, which was conducted with two test systems, one prepared with “river” water and the other with “pond” water. In this first study up to 32.8% AR was detected in the water phase three days after application (“river”), and up to 26.8% AR could still be found 7 days after application. Since the second study clarifies the percentual distribution (peak area) of either metabolite the former study results were set in relation to the results of the actual study. The recalculated distribution of NIR of the former study for day 3 and day 7 revealed that the maximum amount radioactivity, which could be assigned to one distinct peak was determined to be 9.9% AR. The detailed results for this recalculation for all seven metabolites and the two sampling days are represented in Table A7_1_2_2_2-4.
5.2.3	CO_2 formation	Not measured in this study since the purpose of this study is laid on identification and quantification of the degradation products of radiolabeled Preventol CMK.
5.2.4	DT_{50} values	The DT_{50} values determined for the seven metabolites range from 7.0 to

Section A7.1.2.2.2-02 Water/sediment degradation study (02)

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36 days.

6 CONCLUSION

6.1 Conclusion

During the course of the study a new and successful HPLC method was developed for separation of non-identified degradation products of Preventol CMK. From the results of this study it can be concluded that the decomposition of Preventol-CMK gives way to the formation of seven degradation products with a maximum amount of 32.8% AR (sum of non identified metabolites). The results of this analysis were then transferred and applied for a re-evaluation of the most significant results of the former study, which led to the conclusion that none of the seven metabolites exceeded amounts of 9.9% AR, corresponding to 19.8 µg/L CMK equivalents.

Preventol CMK is very fast degraded in aerobic water/sediment systems. The half-lives of the formed metabolites were very short and ranged between 7.0 and 36 days.

6.1.1 Reliability [redacted]

6.1.2 Deficiencies none

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	23/08/2011 [redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]

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

	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 A7_1_2_2_2-1: Characteristics of the water/sediment systems used

Sediment characteristics	
Name	Pond (Kellmetschweiher)
Origin Latitude: Longitude:	Germany Near Böhl-Iggelheim 49°21'N 8°20' E
Sampling date in the field	October 13, 2009
pH at sampling site (water)	8.29 (14°C)
Redox-potential (mV) at sampling site (water)[1)]	176
Oxygen saturation [mg/L] at sampling site (water) [2)]	8.31
Distance from bank [m] (water/sediment) [1)]	~1 / ~1
Sampling depth [cm] (water/sediment) [1)]	~ 30 / ~15
	Sediment parameters[2)]
pH (calcium chloride)	9.8
TC (Total Carbon) [%]	0.08
TOC (Total Organic Carbon) [%]	0.01
TIC (Total Inorganic Carbon) [%]	0.07
Carbonates (Calculated) [%]	0.58
CEC (Mulish method) [mval Ba/100 g]	2.1
N tot. (Titrimetic method) [mg/kg] [2)]	40
P tot. (ICP-OES) [mg/L] [2)]	46.8
Textural class (USDA)	Sand
Textural analysis < 2 µm clay [%] 50-2 µm silt [%] 2000-50 µm [%]	 1.1 1.8 97.1
Textural class (Din 4220)	Sand
Textural analysis < 2 µm clay [%] 50-2 µm silt [%] 2000-50 µm [%]	 1.1 2.3 96.6
	Water parameters[2)]
pH	7.5
DOC (infrared detection) [mg/L]	14.9
Total Nitrogen (TN_b) (Chemoluminescence) [mg/L]	< 1
Total Phosphorus (ICP-OES) [mg/L]	0.05
Water Hardness (Titrimetric method) [°dH] [1)]	5.82

[1)]: determined by the Test Facility,
[2)]: determined by Chemisches Institut Pforzheim GmbH CIP; Dr. Rainer Kiefer; Feb.2010

Table A7_1_2_2_2-2: Presentation of the seven detected metabolites of ^{14}C-Preventol CMK in the water phase, their retention times and the peak area distribution after application of both HPLC-methods. Mean values are expressed in percentage of the radioactivity applied.

Metabolite	n.i.-1	n.i.-2	n.i.-3	n.i.-4	n.i.-5	n.i.-6	n.i.-7
Retention time (min)	1.1 - 1.3	1.9 – 2.5	4.5 – 4.7	10.0 – 10.2	11.9 – 12.0	12.9 – 13.1	18.6 – 18.9
Peak Area (%)							
HPLC run 1	9.1	29.0	2.9	31.7	7.4	6.9	13.1
HPLC run 2	11.8	23.6	9.8	28.9	7.2	8.2	10.5
Mean value (Total Peak Area = 100%)	10.5	26.3	6.4	30.3	7.3	7.6	11.8

Table A7_1_2_2_2-3: ^{14}C-Preventol CMK: Presentation of the relationship between the total amount of NIR on day 7 (22.8% AR) and the results of HPLC-analysis regarding a total peak area of 100%

Metabolite	n.i.-1	n.i.-2	n.i.-3	n.i.-4	n.i.-5	n.i.-6	n.i.-7
Retention time (min)	1.1 - 1.3	1.9 – 2.5	4.5 – 4.7	10.0 – 10.2	11.9 – 12.0	12.9 – 13.1	18.6 – 18.9
[% AR]							
Day 7	2.4	6.0	1.5	6.9	1.7	1.7	2.7

Table A7_1_2_2_2-4: Evaluation of the outcome of the former aquatic study of Preventol CMK by using the results of the new HPLC method of sampling day 7 performed in the actual water/sediment study

HPLC results of the actual study using the separated HPLC fraction containing non-identified metabolites of day 7 [% Peak Area]							
Metabolite	n.i.-1	n.i.-2	n.i.-3	n.i.-4	n.i.-5	n.i.-6	n.i.-7
Peak Area [%]	10.5	26.3	6.4	30.3	7.3	7.6	11.8
HPLC/LSC results of the former study charged against HPLC results of the actual study [% AR]							
Day 3 [1)] **(32.8% AR)**	3.4	8.6	2.1	9.9	2.4	2.5	3.9
Day 4 [3)] **(27% AR)**	**2.8**	**7.1**	**1.7**	**8.2**	**2.0**	**2.1**	**3.2**
Day 7 [2)] **(26.8% AR)**	2.8	7.0	1.7	8.1	2.0	2.0	~~1.0~~ **3.2**
Day 35 [4)] **(2.4% AR)**	**0.3**	**0.6**	**0.2**	**0.7**	**0.2**	**0.2**	**0.3**
Day 35 [5)] **(17.8% AR)**	**1.9**	**4.7**	**1.1**	**5.4**	**1.3**	**1.4**	**2.1**

1) Mean value of the sample containing the maximum amount of NIR ever measured in the former study (test system river)

2) Mean value of the sample ~~containing the maximum amount of NIR~~ measured in the former study on the same sampling day as in the actual study (test system pond)

3) **Mean value of the sample containing the maximum amount of NIR ever measured in the former study (test system pond)**

4) **Mean value of the sample measured in the former study at the end of test (test system river)**

5) **Mean value of the sample measured in the former study at the end of test (test system pond)**

Figure A7_1_2_2_2-1: HPLC chromatogram of the separated and concentrated water phase (day 7).

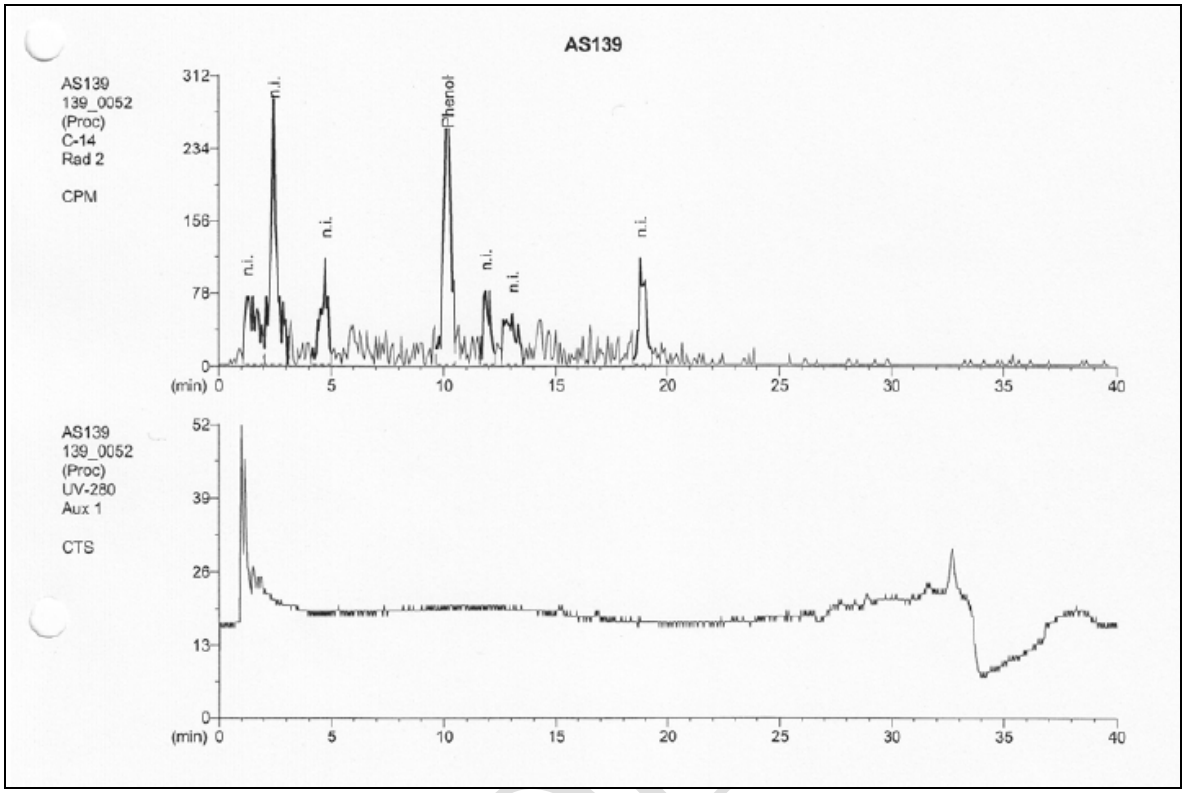


Table A7_1_2_2_2-5: Peak Area Report from the HPLC chromatogram of the separated and concentrated water phase (day 7) (Note: The values reported below origin from a repeated HPLC analyses due to missing Peak Area Reports from the first analysis run)

Peak Area Report (% Pks)					
Name	Retention Time (min.)	Channel 1 - C-14 CPM			
		Pk#	Area	% Pks	Conc.
n.i.	1.27	1	696	11.84	0.00
n.i.	2.47	2	1386	23.57	0.00
n.i.	4.73	3	576	9.80	0.00
Phenol	10.20	4	1698	28.88	0.00
n.i.	11.87	5	426	7.24	0.00
n.i.	13.07	6	480	8.16	0.00
n.i.	18.80	7	618	10.51	0.00
Total Peak Area:			5880		
Total Run Area:			9168		

Figure A7_1_2_2_2-2: HPLC co-chromatogram of the separated and concentrated water phase (day 7) showing identical retention times of n.i.-4 and phenol

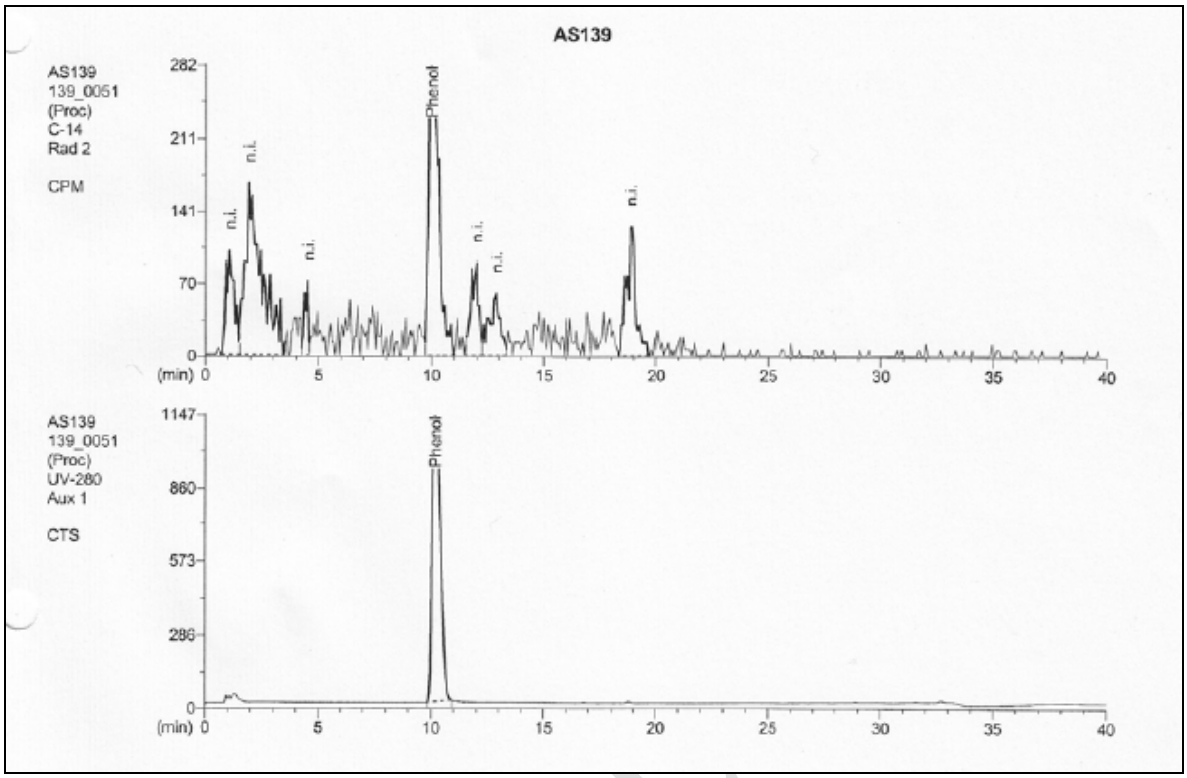
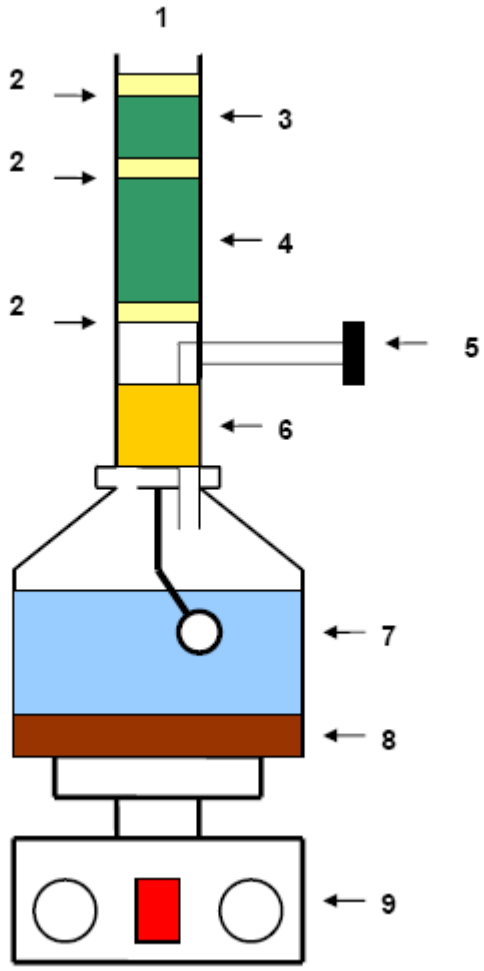


Table A7_1_2_2_2-6: Peak Area Report from the HPLC co-chromatogram of the separated and concentrated water phase (day 7) showing identical retention times of n.i.-4 and phenol (Note: The values reported below origin from a repeated HPLC analyses due to missing Peak Area Reports from the first analysis run)

Peak Area Report (% Pks)									
Name	**Retention Time (min.)**	**Channel 1 C-14 CPM**				**Channel 2 UV-280 CTS**			
		Pk#	**Area**	**% Pks**	**Conc.**	**Pk#**	**Area**	**% Pks**	**Conc.**
n.i.	**1.07**	**1**	**642**	**9.08**	**0.00**				
n.i.	**1.93**	**2**	**2052**	**29.01**	**0.00**				
n.i.	**4.53**	**3**	**204**	**2.88**	**0.00**				
Phenol	**10.00**	**4**	**2244**	**31.72**	**0.00**				
Phenol	**10.17**					**1**	**14761**	**100.00**	**0.00**
n.i.	**12.00**	**5**	**522**	**7.38**	**0.00**				
n.i.	**12.87**	**6**	**486**	**6.87**	**0.00**				
n.i.	**18.87**	**7**	**924**	**13.06**	**0.00**				
Total Peak Area:			**7074**				**14761**		
Total Run Area:			**10530**				**37659**		

Figure A7_1_2_2_2-3: Test vessel connected with the trapping system



1. Glas tube (total length 14.5 cm),

2. Quartz wool (0.2 g),

3. Soda lime (4 g), adsorbent for atmospheric $^{14}CO_2$,

4. Soda lime (10 g), adsorbent for atmospheric $^{14}CO_2$,

5. Closed tube for purging the incubation vessel,

6. Oil wetted quartzwool, adsorbent of volatile organic compounds (1g, wetted with 2% paraffin oil soluted in hexane),

7, 8, 9. Aquatic metabolism flask containing sediment (8) and water layer (7); water stirred gently by magnetic stirrer (9); flask inner-diameter 10 cm; surface area 78.5 cm²

Section 7.1.2.2.2-03 Water/sediment degradation study (3)

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Reference

Dixon, E.M., 1997: Proposed environmental quality standards for 4-chloro-3-methyl-phenol in water. Draft final report to the Department of the Environment, UK. 72p.

Bolz, U. et al., 1999: Determination of phenolic xenoestrogens in sediments and sewage sludges by HRGC/LRMS. Organohalogen Compounds, Vol. 40, 65-68.

Bolz, U. et al., 2001: Phenolic xenoestrogens in surface water, sediments, and sewage sludge from Baden-Württemberg, south-west Germany. Environmental Pollution, 115, 291-301.

Körner, W. et al., 2001: Steroid analysis and xenosteroid potentials in two small streams in southwest Germany. Journal of Aquatic Ecosystem Stress and Recovery, 8, 215-229.

Lacorte, S. et al., 2001: Main findings and conclusions of the implementation of Directive 76/464/CEE concerning the monitoring of organic pollutants insurface waters (Portugal, April 1999 – May 2000). Journal of Environmental Monitoring, 3, 475-482.

Schmidt-Bäumler, K., et al., 1999: Occurrence and distribution of organic contaminants in the aquatic system in Berlin. Part II: substituted phenols in Berlin surface water.

Studies summary

Bibliographical monitoring data are available for surface water and The samplings have been carried out at in Germany, UK and Portugal. The results are summarised below and in the following table.

In Germany, analyses of sediments from two small streams and the lake Constance were carried out by Bolz et al. (1999). CMK was not present above the detection limit in Lake Constance sediment and could only be found in one of two stream sediments (0.002 mg/kg dwt). Further analyses have been performed in the river where CMK has been detected in the sediment (Bolz et al., 2001; Körner et al., 2001, Baden-Württemberg, South West Germany). The river Körsch (57E+06 m^3/a) receives effluents from six STPs (e.g. 20E+06 m^3/a). Concentrations in water and sediment of this river have been compared to other rivers in the same region. Water samples were taken over 8 days in June 1998 whereas sediment samples were collected between 1996 and 1999 from the river bank (0-4cm). Regarding surface water samples, CMK could only be detected in the river Körsch however, below the quantification limit of 0.010 µg/L. It was detectable in the sediment samples from 5 of 7 rivers, mainly at concentrations equal or below 2 µg/kg. The highest sediment concentration (15 µg/kg) was measured in the river Körsch.

Schmidt-Bäumler et al. (1999) collected 30 representative surface water samples from sewers, rivers, canals and lakes in Berlin and analysed them for the presence of 22 substituted phenols, amongst CMK. The purpose of this screening was to monitor the impact of the sewage effluents on the surface water quality and to identify possible sources for ground- and surface water contaminants. Samples were taken above and below points where sewage effluents were discharged into the surface water. Whenever possible, the samples were collected from the middle of the watercourses at a depth of 2 meters. CMK was found in 22 of the 30 water samples at concentrations between 0.05 µg/L and 0.14 µg/L. A

Section 7.1.2.2.2-03 Water/sediment degradation study (3)	
Annex Point IIIA, XII.2.1.	
	significant correlation between the input of sewage effluents and the CMK concentration could not be established. Dixon (1997) summarised monitoring data ascertained by the United Kingdom Environment Agency in 1995 regarding CMK concentrations in fresh, ground and marine waters in the Midlands and North West regions in England. The results are summarised in ***Erreur ! Source du renvoi introuvable.***. Considering surface waters, 96% (n = 1596) of all analysed samples did not contain CMK above the limit of detection (LOD = 0.2 to 5 µg/L). Residues in the remaining surface water samples (n = 66) varied between 0.5 – 6.9 µg/L for the Midland and 0.7 to 6.6 µg/L for the North West region. In almost half (n = 32) of the samples containing CMK, residues were below 1.0 µg/L. Further information provided by the Midland region on identification of CMK roots revealed different diffuse contaminated land sources: remains of steel works, an assortment of urban residential and industrial estates, sewer overflows, public highways, old mining areas and an underground fire. Elevated concentrations were also due to problems that occurred during the installation of a new effluent treatment plant at a paper mill. Groundwater samples did not contain CMK above the LOD. Only one result reported for marine waters is greater than the LOD, this was a concentration of 0.6 µg/L reported in an estuary in the North West region. Lacorte et al. (2001) briefed the results of a monitoring program carried out in Portugal from April 1999 to May 2000. Altogether 644 surface water samples from 46 sites were analysed for organic and organotin compounds as well as heavy metals. Water samples were collected from the river middle bed. CMK was detected in 64 of 632 surface water samples (LOQ ≤ 0.1 µg/L), and over 0.1 µg/L in 51 samples, corresponding to 8.1% of total samples. In 49 samples the substance was present at concentrations between 0.1 and 1.0 µg/L whereas 2 samples contained the compound at a higher concentration than 1.0 µg/L. In summary, the monitoring data support the argument that CMK will not persist in aquatic systems.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15/06/12
Evaluation of applicant's justification	[redacted]
Conclusion	
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*

Section 7.1.2.2.2-03	**Water/sediment degradation study (3)**
Annex Point IIIA, XII.2.1.	
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	

Table 1: Monitoring data on the occurrence of CMK in surface water and sediment

Sampling site/ location	Date of sampling	Limit of quantification/ determination (µg/L or µg/kg)	Surface water concentra-tion (µg/L)	Sediment concentration (µg/kg d.m.)	Reference
Sewer Schönlinde and Münchehofe	16. and 19.09. 1996; n = 2	0.015 (LOD) 0.050 (LOQ)	n.d.	n.m.	Schmidt-Bäumler et al. (1999)
Ditch Buchholz and Rosenthal	16.09.1996; n = 2		n.d.	n.m.	
River Wühle	16.09.1996; n = 3		n.d.	n.m.	
River Erpe	19.09.1996; n = 2		< 0.05 / 0.12	n.m.	
River Spree	19. and 26.09.1996; n = 3		< 0.05 – 0.06	n.m.	
River Dahme	19.09.1996; n = 1		n.d.	n.m.	
River Havel	24. / 26.09.1996; n = 4		< 0.05 – 0.11	n.m.	
River Panke	26.09.1996; n = 2		< 0.05 / 0.07	n.m.	
Canal Teltow	24.09.1996; n = 7		0.07 – 0.14	n.m.	
Canal Landwehr	26.09.1996; n = 1		0.09	n.m.	
Lake Müggelsee	19.09.1996; n = 1		0.12	n.m.	
Lake Wannsee	24.09.1996; n = 1		< 0.05	n.m.	
Lake Tegeler See	26.09.1996; n = 1		0.06	n.m.	
River Körsch	n.r., n = 1	n.r.	n.m.	2	Bolz et al. (1999)
River Sulzbach	n.r., n = 1		n.m.	n.d.	
Lake Constance	n.r., n = 1		n.m.	n.d.	
River Körsch	Sw: June 1998; n = 8 Sed: 1996-1999: n = 3	Sw: <0.01–0.05 (LOD)/ 0.01–0.05 (LOQ) Sed: ≤0.5 (LOD)/ 0.6 – 2 (LOQ)	n.d. - < 0.010	1 - 15	Bolz et al. (2001), Körner et al. (2001)
River Krähenbach	Sw: June 1998; n = 9 Sed: 1996-1999: n = 2		n.d.	n.d. / 1	
River Danube	Sw: 1998, 1999; n = 3 Sed: 1996-1999: n = 1		n.d.	2	
River Erms	Sw: 1998, 1999; n = 2 Sed: n.m.		n.d.	n.m.	
River Neckar	Sw: 1998, 1999; n = 1 Sed: 1996-1999; n = 2		n.d.	n.d.	
River Sulzbach	Sw: n.m. Sed: 1996-1999; n = 1		n.m.	2	
River Echaz	Sw: n.m. Sed: 1996-1999; n = 1		n.m.	n.d.	
River Braunsel	Sw: n.m. Sed: 1996-1999; n = 1		n.m.	1	

Table 1 (cont.): Monitoring data on the occurrence of CMK in surface water and sediment

Sampling site/ location	Date of sampling	Limit of quantification/ determination (µg/L or µg/kg)	Surface water concentration (µg/L)	Sediment concentration (µg/kg d.m.)	Reference
Rivers and brooks in Midland and North West regions, UK	1995; n = 1662	LOD = 0.2 - 5	n.d. (n = 1596) Midland: < 1.0 (n = 28) 1.0 – 6.9 (n = 27) North West: < 1.0 (n = 4) 1.0 - 6.6 (n = 6)	n.m.	Dixon (1997)
Groundwater samples in Midland and North West regions, UK	1995; n = 125		n.d.	n.m.	
Marine water samples in the North West region, UK	1995; n = 2		n.d. / 0.6	n.m.	
Surface water samples from 46 sites in Portugal	Monthly, April 1999 – May 2000; n = 632	LOD ≤ 0.1	n.d. (n = 568) < 0.1 (n = 13) 0.1 – 1 (n = 49) > 1 (n = 2)	n.m.	Lacorte et al. (2001)

n.m. = not measured; d.m. = dry matter; sw = surface water; sed = sediment; sludge = sewage sludge; d.m. = dry matter; n.d. = not detectable; LOD = limit of determination. LOQ = limit of quantification

Section A7.1.3 Adsorption / desorption screening test (01)

Annex Point IIA, VII.7.7

Official use only

		1 REFERENCE
1.1	**Reference**	Erstling, K. and Feldhues, E. (2001): Adsorption/Desorption Bayer AG, ZF – Zentrale Analytik, Leverkusen, Germany, Report No. A 01/0108/05 LEV, unpublished, Date: 2001-09-13; amended: 2001-11-13 and 2007-02-22 Knopf (2001): Analytical characterisation Bayer AG, ZF – Zentrale Analytik, Leverkusen, Germany, Report No. A 01/0108/00 UER, unpublished, Date: 2001-08-29
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes Proposal for new OECD test guideline 121, 2001
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]

Section A7.1.3 Adsorption / desorption screening test (01)

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3.1.4	Further relevant properties	-
3.1.5	Method of analysis	Identity of test substance: NMR A small amount of the substance was dissolved with dimethyl sulfoxide d6, and tetramethylsilane was added as standard of the chemical shift. The 1H-, 13C (proton-decoupled)-and 13C DEPT (proton decoupled) spectra were recorded. The identity was confirmed by comparison with reference data. Assay and by-products of the test substance: GC To about 100 mg of the sample, 1 mL N-Methyl-N-trimethylsilyltrifuoracetamide is added and allowed to react for about 25 min at about 80°C. GC parameter: Column: quartz capillary, length 50 m, inner diameter 0.32 mm, coating CP 5 CB, film thickness: 1.2 µm Temperature: 120 to 280°C with 8K/min and 20 min 280°C Detection: FID, temperature 250°C Injection volume: 0.001 mL Quantification: area-% without peak areas of the derivatization agent Test for adsorption: HPLC HPLC parameter: Column: LiChrospher 100 CN, 5 µm, 250 x 4 mm; Mobile phase: 400 mL acetonitrile : 85 mL buffer solution pH 6 : 515 mL water Flow rate: 1.0 ml/min, isocratic Column temperature: 40°C Detector: UV, 220 nm Injection volume: 5 µl
3.2	**Degradation products**	Degradation products tested: No (HPLC screening test)
3.2.1	Method of analysis for degradation products	Not relevant
3.3	**Reference substance**	Calibration substances for assessing the adsorption: Sodium nitrate (assessment of the dead time of the HPLC system) 2-Nitrobenzamide N,N-Dimethylbenzamide Acetanilide Naphthalene 1,2,3-Trichlorobenzene Fenthion
3.3.1	Method of analysis for reference substance	HPLC analysis (see Point 3.1.5)
3.4	**Soil types**	Not relevant (HPLC method)
3.5	**Testing procedure**	
3.5.1	Test system	The adsorption coefficient of p-chloro-m-cresol was determined by reversed phase chromatography. Sodium nitrate was used to determine

Section A7.1.3 Adsorption / desorption screening test (01)

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		the HPLC system's dead time. From the retention times of the reference substances and the dead time of the HPLC column, a capacity factor for each of the substances was calculated. The retention time and capacity factor of the test substance was brought into relation to those of the reference substances. The test is based on the proposal for a new test guideline OECD 121, 2001.
3.5.2	Test solution and Test conditions	About 100 mg of the dead time marker sodium nitrate was dissolved in acetonitrile/water. About 100 to 170 mg of the reference substance and the test item were each dissolved in acetonitrile/water. For a solution with the dead time marker, all reference substances and the test substance 1 mL each of the single solutions were dissolved with acetonitrile to 100 mL. The concentration of the test substance in the mixture solution was about 10 mg/L
3.6	**Test performance**	
3.6.1	Preliminary test	Not performed
3.6.2	Screening test: Adsorption	HPLC screening test according to a proposal for a new guideline OECD 121, 2001
3.6.3	Screening test: Desorption	Not performed
3.6.4	HPLC-method	See Point 3.1.5
3.6.5	Other test	No
		4 RESULTS
4.1	**Preliminary test**	Not performed
4.2	**Screening test: Adsorption**	The results are summarized in Table A7_1_3-1
4.3	**Screening test: Desorption**	Not performed
4.4	**Calculations**	$k' = (t_r - t_0)/t_0$ (for parameter see Table A7_1_3-1) Log k' = a + b log Koc Linear regression gives: a = -0.48334 b = 0.33215 r = 0.97979 The adsorption coefficient of the test substance was calculated as: log Koc = 2.2 (Koc = 158.5)
4.5	**Degradation product(s)**	Not relevant (see Point 3.2)

Section A7.1.3 Adsorption / desorption screening test (01)

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A HPLC screening test for assessing the adsorption properties of p-chloro-m-cresol was conducted according to a proposal for a new guideline OECD 121, 2001. The test system is described under Point 3.5.1. No relevant deviations from the guidelines occurred.
5.2	**Results and discussion**	The dead time (t_0) with sodium nitrate is 1.652 min. The linear regression of measured k' against Koc values yielded a line with a slope of 0.33215, an intercept of -0.48334 and a correlation coefficient of $r^2 = 0.97979$. The adsorption coefficient of the test substance was calculated as : log Koc = 2.2 (Koc = 158.5) Conclusive information is given in Table A7_1_3-1.
5.2.1	Adsorbed a.s. [%]	Not relevant (HPLC screening test)
5.2.2	K_a	Log k' = 0.251
5.2.3	K_d	Not relevant (HPLC screening test)
5.2.4	Ka_{oc}	log Koc = 2.2 (Koc = 158.5)
5.2.5	Ka/Kd	Not relevant (HPLC screening test)
5.2.6	Degradation products (% of a.s.)	Not relevant (see Point 3.2)
5.3	**Conclusion**	Based on the results of this screening test and taking into consideration the classification system according to Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973), compounds having Koc values within the range of 130-690 are considered to be of low mobility and those having Koc values > 690 are considered to be immobile. The test substance p-chloro-m-cresol can be assumed to be of low mobility in soils.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_1_3-1:Retention times and Koc values for p-chloro-m-cresol and reference substances

Substance	Retention time (t_r) in min*	Capacity factor k'*	Log k'	Log Koc
2-Nitrobenzamide	3.002	0.818	-0.087	1.45
N,N-Dimethylbenzamide	3.298	0.997	-0.001	1.52
Acetanilide	3.391	1.053	0.022	1.25
Naphthalene	6.479	2.923	0.466	2.75
1,2,3-Trichorobenzene	7.121	3.312	0.520	3.16
Fenthion	8.929	4.406	0.644	3.31
p-Chloro-m-cresol	4.597	1.783	0.251	2.21

* mean value form 3 single values; the dead time (t_0) with sodium nitrate is 1.652 min

Section 7.1.4.1 **Annex Point IIIA 12.2**	**Field study on accumulation in the sediment**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	[redacted]	
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	27/10/11	
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 7.2.1 **Annex Point IIIA 12.2.2**	**Fate and behaviour in soil** **Aerobic degradation in soil, initial study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	
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	26/12/2011	
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		

Annex I : Bilateral discussions with the Applicant about the requirement of additional data on the fate of CMK in soil

Statement on the anaerobic/aerobic degradation of Chlorocresol (CMK) in soil with special emphasis on the persistency criteria and the extent of soil exposure – Update Oct. 18, 2011

In the course of the review of the dossier for the a.s. *p*-Chloro-*m*-cresol (CMK, 4-chloro-3-methylphenol, chlorocresol; CAS 59-50-7) notified for the BPD Product Types 1, 2, 3, 6, 9 and 13 we like to further expand on the request for a biodegradation study of CMK in soil.

The main argument delivered by RMS for requesting a complete study on biodegradation of CMK in soil (including quantification of bound residues and possible metabolites) is based on the need for assessing a potential persistency of the a.s. or its metabolite(s) in soil according to Annex VI of the BPD. Together with the following statement the applicant delivers additional information (several documents are submitted with this argumentation) which approves that the submission of a complete biodegradation study in soil including identification and determination of breakdown products (OECD GL 307) is not considered necessary. This conclusion is drawn since the biodegradation extent and rate constants of CMK disprove the persistency of the substance in soil and further more because exposure of soil is considered to be insignificant (due to several degradation and dissipation processes taking place beforehand the substance might reach the terrestrial compartment).

The criterion for persistency in soil according to Paragraph 85 in Annex VI of the BPD stressed by RMS defines that a substance which is not degraded in soil during field testing over a period of one year and/or a substance that forms non-extractable residues in amounts exceeding 70% of the initial dose after 100 days with a mineralization rate of less than 5% in 100 days shall not be authorised by Member States when unacceptable contamination of soil is likely to occur. With respect to the environmental fate of CMK in aerobic soil it can be doubtlessly concluded from several publicised studies on biodegradation that CMK disappears rapidly with half-life values ranging between 4.1 and 21 days for aerobic degradation of CMK in soil (Sattar 1989, Loehr & Matthews 1992). Both authors prove (primary) degradation of the test substance, but do not identify potential breakdown products and their environmental fate in soil. The results of Loehr & Matthews (1992) have recently been confirmed in an aerobic biodegradation study by Nitsche (2011) which was submitted in summer 2011 in order to be included into the BPD dossier. Nitsche reports a half-life of 4.4 days for a certified sandy silt loam soil at an application rate of approx. 10 mg chlorocresol/kg soil. In the study the dissipation of the parent compound was analytical monitored by HPLC methodology. For the rapid dissipation at least primary degradation of the chlorocresol must be assumed.

As explained in the document “Fate and Toxicity of potential CMK metabolites in soil_27122010” the degradation pathway of CMK can be described by referring to the commonly known degradation scheme for phenolic compounds (Phenol, BUA Report 209 from May 1997, p.142). According to this

degradation scheme phenolic compounds are first oxidised (by formation of catechol as a transient product) before cleavage of the aromatic ring structure occurs leading to the formation of carbonyl and carbonic acid structures (as polar substances), which are rapidly further degraded either to CO_2 or react chemically with the organic matter in the soil. For clarification on the mineralization rate of CMK and the quantification of potentially built non-extractable residues (NERs) scientific read across as established under REACH may be performed (cross-reading for biodegradation studies in soil are scientifically justified as both compounds are phenolic structures and both chemicals show a ready biodegradation in aquatic medium).

Hence, relevant results from a study by Thomas W. Federle (1988) on the mineralization of phenol, benzoic acid and benzylamine in soil followed by $^{14}CO_2$ evolution are laid down in the following. Analysis of the mineralization of aromatic compounds is performed as a function of depths in two 20-m sandy soil profiles (vadose and saturated zones). Collected soil samples from two different sites in north central Wisconsin and various depths were adjusted to 20-25% water content and spiked with 50 ng/g soil of the ^{14}C-ring-labeled compounds. The first-order rate constant for phenol mineralization was determined by non-linear regression for several soil depths and ranged between 0.31 and 3.3 d^{-1} with the average being 1.2 d^{-1} (= mean half-life of 0.56 days). Degradation rates in the saturated zone were comparable with those in the vadose zone. The degradation pathway for Phenol is oxidation to catechol with subsequent *meta* or *ortho* cleavage of the ring. Mineralization of phenol and the other two phenolic compunds occurred in all samples without a lag-period. With regard to the recovery of $^{14}CO_2$ it is shown that phenol is mineralised between 17 and 32% of applied RA during 64 days of incubation (Federle 1988). Compared with the recovery rates of benzoic acid and benzylamine (up to 53%) this reduced mineralization extend can be explained by volatilisation of the compound due to its high vapour pressure. After the incubation period of 64 days the radioactivity (RA) remaining in the test systems was fractionated. In the biotic samples (called “live samples” by the author in contrast to the abiotic controls) for phenol 91 – 95% of the residual RA was not extractable with water. In the abiotic controls (containing formalin) the non-extractable residues accounted for 57 – 29 % of the residual RA indicating that the additionally bound fraction of phenol in the live samples likely refers to the amount associated with the insoluble organic matter. Total recoveries of radioactivity from these samples were 60-72% for phenol. Figure 3 of the publication illustrates the results:

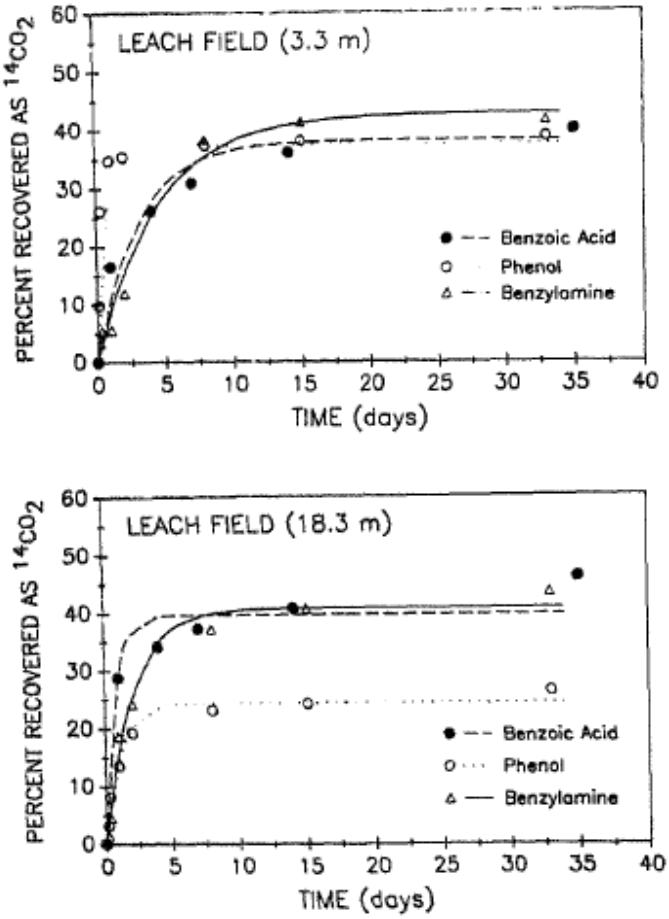

FIG. 3. Percent of radiolabeled benzoic acid, phenol, and benzylamine recovered as $^{14}CO_2$ over time in representative samples from 3.3 (top) and 18.3 m (bottom) in the leach-field profile. The curves show the fitted first-order functions.

Federle (1988) as well as Subba-Rao and Alexander (1982) state that the mineralization extend of aromatic substances becomes less when sorption/incorporation of the polar breakdown products (carbonyl and carbonic acid structures) into humic material becomes greater. From these study results the following conclusions can be drawn for the environmental fate of phenol in soil:

Degradation of phenol takes place with an average half-life of 0.56 days, whereas the ultimate degradation to $^{14}CO_2$ accounts to 17 and 32% during 64 days. An additional not quantified amount of phenol is assumed to volatise from soil, and a maximum fraction of 68.7% of AR (95.5% of AR bound to soil particles from 72% of maximum total AR recoveries in the test systems after incubation) might in a worst-case situation be bound to the soil compartment which constrains bioavailability. The discrepancy between the water-extractable portion of RA in biotic and abiotic samples indicate that breakdown products of phenol are incorporated into biomass (as humic acids) as demonstrated by Stott *et al.* (1983) for catechol being the primary breakdown product of phenol. Federle (1988) proves in his study that the analysed aromatic compounds are rapidly degraded by microbes in subsurface environments, which is properly due to preadaptation for utilization of natural aromatic substrates. Preadaption is very likely a consequence of the fact that a high proportion of humic materials consists

of aromatic moieties. Moreover, the author concludes that the metabolism pathways for the three analysed aromatic compounds can be described to follow common or parallel schemes. When finally comparing the data described above for the degradation of phenol in soil with the persistency criterion as described in Annex VI of the BPD it can be derived by cross-reading that also CMK and its breakdown products may be mineralised to more than 5% in 100 days. Therefore, the parent as well as potential breakdown products can be concluded not to fulfil the persistency criterion.

To support the ultimate degradation and mineralization of chlorocresol in soil further read-across may be taken into consideration: the scientific publications by Haider et al. (1974) and Weijnen et al. (1989) investigate the mineralisation rate (measured via $^{14}CO_2$ evolution) of phenol and/or chlorophenols under aerobic conditions in soil. The studies support the assumption that simple phenolic compounds as 4-chloro-3-methylphenol (Chlorocresol) should be mineralized for more than 5% in 100 days:

The study performed by Haider et al. (1974) on the mineralization of ^{14}C-labelled chlorinated aromatic derivates in a natural soil from Flachstöckchenheim near Braunschweig, Germany (1.26% C; 0.12% N; pH(KCl) 7,1) by soil bacteria revealed mineralisation rates ranging between 25 and 65% for mono-, di- and trichlorinated phenolic substances after 10 weeks. Most extensive degradation rates after 10 weeks were found for phenol (65% CO_2), followed by a mixture of 2,4,6/2,4,5-Trichlorphenol (51% CO_2), and a mixture of 2,4/2,6-Dichlorophenol (48% CO_2). 4-Chlorophenol as a structurally related compound to chlorocresol regarding the para-position for the chloride molecule proved CO_2 evolution up to 35%; 2-Chlorophenol showed a mineralisation extent of 25% (Haider et al. 1974, Table 3). Furthermore, preliminary degradation studies showed that soil samples from different origins revealed similar abilities for degradation of the contemplated aromatic derivates.

From Haider et al. 1974, Table 3: evolution of labelled CO_2 – data for the phenolic compounds

COMPOUND	3 Days	1 Week	2 Weeks	5 Weeks	10 Weeks
Phenol	45,5	48	52	60	65
2-Chlorphenol	7,5	13	14,7	21	25
4-Chlorphenol	15,4	22,2	24	31	35
Dichlorphenol	1,4	31,4	35	43	48
Trichlorphenol	1,6	35	38	47	51

A similar mineralization extent for 4-Chlorophenol by aerobic soil bacteria was detected by Weijnen et al. (1989) in a soil typ from The Netherlands. Their experimental research with C-labelled 4-chlorophenol in Rolde soil (3.3% org. C; pH(KCl) 5,8) showed a CO_2 evolution of 18% within 6 weeks (44 days) of study duration. The mineralisation extent for 3,4-Dichlorphenol in the same soil type accounted for 13% within 41 days. The data as presented in tables 5.8 and 5.9 of the study report is copied below:

Table 5.8: break-up of radioactive labelled 4-monochlorophenol and formation of labelled CO_2 in Rolde soil. Percentage recovery related to the quantity of added radioactivity.

		AEROOB		STERILE	
SAMPLE	TIME (DAYS)	REC-CF (%)	REC-CO_2 (%)	REC-CF (%)	REC-CO_2 (%)
1	0	83.26	0.02		0.15
2	0	77.17	0.53	98.54	0.00
3	0	90.20	0.20	95.94	0.05
4	1	63.47	1.35	95.16	0.01
5	1	72.36	0.88	92.79	
6	1	70.18	1.78	93.13	
7	3	49.43	0.18	95.63	0.00
8	3	60.30	4.04	96.77	
9	3	62.54	4.14	99.72	
10	7	39.52	9.01	94.61	0.03
11	7	4.33	19.93	95.33	
12	7	39.60	9.25	94.72	
13	10	6.47	20.47	96.09	0.03
14	10	25.41	13.86	98.53	
15	10	23.20	7.50	95.78	
16	14	3.74	23.30	95.17	0.01
17	14	4.59	20.90	97.31	
18	14	3.51	22.51	93.13	
19	21	3.27	21.86	99.04	0.03
20	21	4.82	16.31	90.77	
21	21	2.87		100.24	
22	44	4.48	17.42	89.85	0.02
23	44	4.49	17.59	92.65	0.02

Table 5.9: break-up of radioactive labelled 3,4-dichlorophenol and formation of labelled CO_2 in Rolde soil. Percentage recovery related to the quantity of added radioactivity.

		AEROOB		STERILE	
SAMPLE	TIME (DAYS)	REC-CF (%)	REC-CO_2 (%)	REC-CF (%)	REC-CO_2 (%)
1	0	87.27	0.03		0.01
2	0	89.32	0.03	95.37	0.01
3	0	86.44	0.03	95.38	0.01
4	3	82.71	0.95	93.66	0.00
5	3	83.75	0.92	93.79	
6	3	81.93	0.88	91.74	
7	7	71.79	2.00	91.44	0.01
8	7	75.15	1.95	93.08	
9	7	70.59	1.30	92.72	
10	14	39.74	4.51	92.68	0.01
11	14	49.92	3.75	91.84	
12	14	34.46	2.65	90.78	
13	22	22.81	9.31	97.04	0.01
14	22	21.17	9.88	91.96	
15	22	29.91	8.40	92.86	
16	27	18.10		83.36	0.01
17	27	15.07	11.08	80.49	
18	27	15.66	10.79	80.72	
19	41	11.22			0.02
20	41	10.63	12.45	94.68	
21	41	11.56	13.91	93.46	

The kinetic aspects of aerobic biodegradation of anthropogenic organic compounds by subsurface microbes were investigated by Swindoll *et al.* (1988). By means of radiotracer methods the biodegradation and the $^{14}CO_2$ mineralization of six natural and nine xenobiotic organic compounds, *inter alia* m-cresol, was studied in a slurry experiment using a Oklahoma subsurface sand soil taken from the aquifer at 4.5 to 5.5 m depth. The data was evaluated with both a first-order and a Michaelis-Menten approach. Kinetic data for phenolic substances reveal reasonable turnover times T(n) for $^{14}CO_2$ formation and complete dissipation of the substance. The turnover times are calculated for a normalized concentration of 0.5µg/g; T(n) for Phenol is reported to be 805.9 h, for m-Cresol 1370.3 hours is found showing a rapid dissipation and mineralization. In the relevant tables 2 and 3 only kinetic evaluation parameters are given, no further measured values are provided in the publication.

From Swindell et al. 1988, Table 2 – Data for Phenol:

Table 2. Calculated turnover times (T_n), first-order rate constants (K_1), maximum velocity (V_{max}) and half-saturation constants (K') for natural substrates

Compound	Soil	T_n (h)	Metabolic parameter	K_1 (h^{-1}; $\times 10^{-3}$)	SE ($\times 10^{-3}$)	V_{max} (ng/g/h)	SE	K' (ng/g; $\times 10^4$)	SE ($\times 10^4$)
Phenol	9S2	805.9	Respiration	3.45	0.41	10.10	1.30	5.00	0.00
			Uptake	55.70	7.70	NS	NS	NS	NS
			Total	58.10	7.80	NS	NS	NS	NS

SE, standard error of estimate; NS, not saturated.

From Swindell et al. 1988, Table 3 – Data for m-Cresol:

Table 3. Calculated turnover times (T_n), first-order rate constants (K_1), maximum velocity (V_{max}) and half-saturation constants (K') for xenobiotic compounds

Compound	Soil	T_n (h^{-1})	Metabolic parameter	K_1 (h^{-1}; $\times 10^{-5}$)	SE ($\times 10^{-5}$)	V_{max} (ng/g/h)	SE	K' (ng/g; $\times 10^4$)	SE ($\times 10^4$)
m-Cresol	9S2	1,370.3	Respiration	51.10	2.90	NS	NS	NS	NS
			Uptake	23.40	4.90	NS	NS	NS	NS
			Total	74.50	5.30	NS	NS	NS	NS

SE, standard error of estimate; NS, not saturated.

Another strong argumentation for non-submission of a new and extended study on aerobic degradation in soil using radioactive labelled material is that CMK is proven in three further experiments to disappear effectively by biotic and abiotic processes so that only negligible amounts of the parent could reach either a sewage treatment plant (STP) and subsequently enter the soil compartment *via* sludge application on agricultural land (no direct emissions to soil are expected due to the product types applied for in the dossiers submitted in 2007), or via direct irrigation of stable cleaning waters on agricultural land:

> Vaporization Experiment

The abiotic dissipation of Chlorocresol from surfaces is proven to be highly effective due to volatilisation processes. Hence, after a certain time period only negligible residues of the active substance are left on disinfected surfaces. The applicant conducted a study on the vaporisation behaviour of 4-chloro-3-methylphenol within the product Neopredisan® 135-1 (applied as PT3 product). This study (Gerharz 2011c) which is submitted with this document, shows that CMK evaporates consistently from an inert surface leaving less than 1% of the originally applied amount after 96 hours. When considering the negligible CMK-load to be expected in STP together with the fact that elimination of CMK in a STP is proven by monitoring data to be highly effective (up to 99.9% removal) the potential exposure of soil to CMK residues via sludge is maximum of insignificant extent if not zero. Cleaning waters from disinfected stables which might be applied directly on agricultural land is also not likely to contain CMK as the substance has evaporated from the surfaces during the normal cleaning intervals of several weeks. Thus, the suitability of the phrase

"unacceptable contamination of soil" which is the condition for the relevance of the persistency criteria in Annex VI is not given.

> Degradation in Stable Cleaning Waters

Apart from monitoring data on the effective biodegradation of the a.s. during waste waster treatment revealing elimination rates of up to 99.9% (*cf.* Section 3.3.3.1 in Doc.II-B), it is demonstrated by monitoring data that in a washing water sample resulting from stable spouting of a previously disinfected (laying hen) stable no CMK was present (Gerharz 2011a). The disinfection event (wetting the surface and waiting approx. 12-24 h until dry) took place before new animals were introduced into the stable, i.e. approx. 12 months before the next cleaning step with water was carried out.

Furthermore, it is experimentally shown by the applicant that CMK newly introduced into a stable cleaning water (received from stable spouting of a previously disinfected (laying hen) stable) is rapidly biodegraded in the washing water sample. As described in the Test Report on CMK degradation in a liquid environment (Gerharz 2011a) submitted with this document, no traces of the compound could be detected in the washing water after the cleaning event although the stable was treated with a chlorocresol-containing product previously. So the washing water was spiked with two different concentrations of CMK (5 mg/kg and 10 mg/kg) and the subsequent degradation was monitored by HPLC analysis of the duplicate samples. For both test concentrations a nearly complete degradation reaching less than 1% of the applied amount was determined for CMK in the test period of 8 days. The substance degraded with a half-life of 2-3 days.

> Degradation in Manure

As described in the ESD for PT18 (OECD 2006) and PT3 (EC 2010) potential residues of the a.s. enter the slurry/manure container for storage before application to agricultural land is expected. Thus, further reduction of the CMK content in slurry/manure for PT3 products is possible due to biological degradation in the storage containers. Despite the fact that no official guideline is available for analysis of this degradation pathway the applicant conducted a degradation study by analysing CMK degradation in pork liquid manure under anaerobic conditions. The corresponding Test Report (Gerharz 2011b) is attached to this document. For the test a fresh manure sample received from ChemCon GmbH was submitted to HPLC analysis for determination of possible CMK residues, which could not be detected. Subsequently, the sample was spiked with a CMK-containing solution at a concentration of 3 mg/kg CMK under argon air flow to maintain anaerobic conditions. The sampling schedule for duplicate HPLC analysis of the CMK concentration in manure was at day 0, day 20, day 27 and day 34. After the test period of 34 days the 4-chloro-3-cresol concentration was less than 17% of applied CMK, i.e. Chlorocresol degraded with a half-life of 15 days. Sodium benzoate was used as a control substance in the test at a concentration of 100 mg/kg and revealed an experimental half-life of 3 days for degradation under anaerobic conditions. The test results for Chlorocresol prove that anaerobic storage conditions contribute to a decrease of CMK in liquid manure.

> Necessity of Soil Dissipation Studies for Readily Biodegradable Substances

Besides the fact that in literature CMK is consistently referred to as a biodegradable substance in soil with fast dissipation rates, it should be stated here that the Technical Meeting (TM III-06) hold from the 16th to the 19th of October 2006 decided to justify non-submission of a biodegradation study in soil due to the ready biodegradability of the corresponding substance in water. According to the submitted OECD tests for CMK (Müller 1992; Weyers 2007; Hanstveit & Pullens 1993) it is classified as readily biodegradable under stringent test conditions (implying a high inoculum activity and/or the presence of appropriate inoculum species being able to degrade CMK). Nevertheless, monitoring data and the already submitted water/sediment-study reveal the fast and exhaustive degradation of CMK in aqueous media.

Furthermore, a publication by Struijs et al. (1995) delivers sound arguments on expected soil biodegradation for substances which are readily biodegradable in the aquatic environment. The rational is based on the organism population density which is much higher for soil than in the OECD tests which are used to assess ready aquatic biodegradation.

Summary

Summarizing the presented facts for the active substance CMK the applicant wants to stress that there are reasonable arguments for a scientific-based waiving of a requested radioactive labelled study on biodegradation in soil. The arguments include valid and confirmed data on the fast biodegradation of the a.s. in soil, and cross-reading of results for the biodegradation rate and mineralization extent of (chloro-)phenolic compounds -including m-cresol- in soil: for chlorocresol dissipation in soil is poven to be rapid (half-life approx. 4-5 days) and mineralization is derived to be significantly above 5% in 100 days even if there were bound residues exceeding 70%. Thus, the applicant considers the aspect of persistency and environmental relevance in soil to be extensively answered and disproved.

The second explanation for non-submission of the requested soil degradation study is based on an exposure-driven argumentation proving that the use of CMK in most of the product-types will not lead to any noteworthy environmental exposure of soil to CMK and its transient breakdown products. Since the a.s. reveals a fast and effective biotic and abiotic degradation or dissipation behaviour in several media, most of them are to be passed before CMK enters the terrestrial compartment via e.g. sludge application.

As a third argument it should be taken into consideration that for a wood preservative the Technical Meeting TM III-06 decided in October 2006 that the non-submission of a biodegradation study in soil is justified if for the substance the ready biodegradability in water is shown. Scientific background for such a decision is provided.

Overall, for chlorocresol the request for a new radioactive labelled biodegradation study in soil including identification and determination of breakdown products (OECD GL 307) is considered disproportional for the applicant as it would add only insignificant scientific value for the environmental evaluation of the substance.

Literature:

Federle, T.W. (1988): Mineralization of monosubstituted aromatic compounds in unsaturated and saturated subsurface soils. Can. J. Microbiol. 34: 1037-1042.

Gerharz, T. (2011a): Test Report: Degradation of 4-chloro-3-cresol in a liquid environment (washing water after stable cleaning – stable with laying hens). Lanxess Deutschland GmbH.

Gerharz, T. (2011b): Test Report: Degradation of 4-chloro-3-cresol in pork liquid manure under anaerobic conditions. Lab Report ID: D 2011-10, Lanxess Deutschland GmbH.

Gerharz, T. (2011c): Test Report: Neopredisan 135-1. Vaporisation behaviour of 4-chloro-3-methylphenol from an inert surface (glass petri dish). Lab Report ID: D 2011-22.1.5, Lanxess Deutschland GmbH.

Haider K., Jagnow G., Kohnen R. & Lim S.U. (1974): Abbau chlorierter Benzole, Phenole und Cyclohexan-Derivate durch Benzol und Phenol verwertende Bodenbakterien unter aeroben Bedingungen. In: Arch. Microbiol. 96, 183-200.

Loehr, R.C. & Matthews, J.E. (1992): Loss of organic chemicals in soil. Pure compound treatability studies, Journal of Soil Contamination 1(4), 339-360 (already submitted: A7.2.1(02)).

Nitsche, M. (2011): Biodegradation of Preventol® CMK (4-chloro-3-methylphenol) in soil under aerobic conditions – Test Report, Lanxess Deutschland GmbH, Leverkusen, Germany. Date: 25th July, 2011

Sattar, M.A. (1989): Fate of chlorinated cresols from environmental samples, Chemosphere 19 (8/9), 1421 – 1426 (already submitted: A7.2.1(01)).

Stott, D.E., Martin, J.P., Focht, D.D., and Haider, K (1983): Biodegradation, stabilization in humus and incorporation into biomass of 2,4-D and chlorocatechol carbons. Soil Sci. Soc. Am. J.45: 66-70.

Struijs, J., and van den Berg, R. (1995): Standardized Biodegradability Tests: Extrapolation to Aerobic Environments. In: Wat. Res. 29, 255-262

Subba-Rao, R.V. and Alexander, M. (1982): Effect of sorption on mineralization of low concentrations of aromatic compounds in lake-water sample. Appl. Environ. Microbiol. 44: 659-668.

Swindoll C.M., Aelion C.M., Dobbins D.C., Jiang O., Long S.C. & Pfaender, F.K. (1988): Aerobic biodegradation of natural and xenobiotic organic compounds by subsurface microbial communities. In: Environmental Toxicology and Chemistry, Vol.7, 291-299.

Weijnen P.H.C., v.d.Berg R., v.d. Berg S. (1989): Biodegradatie van chloorfenolen in de bodem. Rapport nr. 728603005, Rijksinstituut voor Volksgezondheid en Milieuhygiene Bilthoven, NL.

Section A7.2.2. Aerobic degradation in soil (03)

Annex Point IIIA, XII 1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Nitsche, M. (2011): Biodegradation of Preventol® CMK (4-chloro-3methylphenol) in soil under aerobic conditions - Test Report, Lanxess Deutschland GmbH, Leverkusen, Germany. Report No.: 2011-07-25 (not published), Date: 25th July, 2011.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No; The adapted test procedure is taken from *Raymond C. Loehr, John E. Matthews 1(4) (1992) 339-360 Journal of Soil Contamination - Loss of organic chemicals in soil: Pure compound treatability studies*	
2.2	**GLP**	No	
2.3	**Deviations**	-	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Preventol® CMK (4-chloro-3-methylphenol)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	Treatment solution was applied to the surface of the soil in each incubation bottle at the application rate of 10 mg Preventol® CMK /kg soil. Following application of the 100 µL to each bottle in five steps a 20 µL to five different points for distribution, the bottles were kept open for a short time allowing volatilization of the methylene chloride. Afterwards the brown glass bottles were closed and stored at room temperature (22°C-23°C) in the dark to prevent photo degradation of the test substance. Before, the moisture content of the soil was adjusted to 80% of field capacity. During the test period the test bottles were weighted every week and the moisture content (moisture loss) was adjusted by adding deionized water if necessary. At zero time, 2, 5, 7, 9, 12, 14, 16 and 19 days after application, a duplicate sample was subject to extraction and HPLC analysis. Extraction was performed with 45 mL Tetrahyrofuran and 5 mL sulphuric acid 0.5M. The samples were stirred for 1 hour before they	

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were extracted in a ultrasonic bath for 30 minutes. For homogenization the samples were stirred again. After sampling of the extracts was done under constant stirring, the extracts were centrifuged (14000 rpm, 7 min) and the supernatants were used for Preventol® CMK analysis by HPLC. The HPLC analysis was done with an external standard.

HPLC- Equipment and conditions

HPLC:	Agilent 1200 series
Rectifying column:	Zorbax Eclipse Plus-C18, 3.5 µm
Manufacturer:	Agilent
Length:	150 mm, internal diameter 3.0 mm
Column temperature:	40°C
Flow:	0.7 mL/min
Eluent A:	demineralized water + 0.04% H_3PO_4 of 85%
Eluent B:	Acetonitrile
Stoptime:	8 min
Posttime:	7 min

Time [*t*] = min	[φ(A)] = %	[φ(B)] = %
0	70	30
6	10	90

Detector:	UV
Wavelength:	200 nm
Gap width:	4 nm
Reference wavelength:	off
Injection volume:	5 µL
Data rate:	< 0.03 min (0.5 s)
Reagents:	- Acetonitrile, quality: gradient of degrees, manufacturer: Merck (article no. 1.00030) - Phosphoric acid 85% industrial union, quality: specially pure Merck (article no.: 1.00563.1000) - Sulphuric acid 0.5M, e.g. AVS Titrinorm Prolabo article no.: 30144.294, E.g. 99+ specially pure, stabilized with BHT, Acros article no. 176630025 - Tetrahydrofuran (THF), E.g. 99+ specially pure, stabilized with BHT, Acros article No.: 176630025

3.2	**Reference substance**	-
3.2.1	Method of analysis for reference substance	-

Section A7.2.2. Aerobic degradation in soil (03)

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3.3 Soil types

The tested soil is a certified mineral soil, which was not disinfected before (supplier: Bayer Crop Science, Monheim, Germany). Sample No.: 08-188024.

Soil type:	Sandy silt loam
pH:	6.9
Total carbon:	1.1%
Humus:	1.9%
clay	12.9%,
sand	53.9%

3.4 Testing procedure

3.4.1 Test system

Rate of aerobic degradation in soil monitored by HPLC analysis (see point 2.1).

3.4.2 Test solution and test conditions

For control reasons the soil was tested on the presence of Preventol® CMK before it was used in the study. In the tested soil Preventol® CMK was ≤ 0.1 ppm. Preparation of the soil included air drying and sieving through a 0.7mm sieve. 10 g of air dried soil was placed in a 100 mL brown glass bottle and the moisture content was adjusted to 80% of field capacity. The bottles were closed and stored at room temperature in the dark for 8 days to allow soil microorganisms to equilibrate to experimental conditions.

A solution of the test substance Preventol® CMK in methylene chloride was prepared in a concentration so that 100 µL of the solution gave the desired mass loading rate of 10 mg Preventol® CMK / kg soil. The mass loading is based on toxicity screening result according to (Lit.:) Raymond C. Loehr, John E. Matthews 1(4) (1992) 339-360.

The 100 µL were added to each bottle with a 20-µL-pipette in five steps to five different points in the soil surface to distribute the solution. Before closing the bottles volatilization of the methylene chloride was allowed for a brief time. Then the brown glass bottles were closed and stored at room temperature (22°C - 23°C) in the dark to prevent photo degradation of the added test substance.

Weekly weighing of the test bottles was performed for determination of the moisture content (moisture loss). When necessary it was adjusted by adding deionized water.

Control experiments with the used soil also included the testing of the recovery rate of Preventol® CMK. In concentration of 10mg CMK / kg soil the recovery rate was 100%; in a concentration of 5mg CMK / kg soil the recovery rate was 101% and in a concentration of 1mg CMK / kg soil the recovery rate was 107%.

4 RESULTS

4.1 Aerobic soil degradation

For details on the decline of Preventol® CMK concentrations in soil for the specific sampling events please see Table A7_2_2_1-2 and Figure A7_2_2_1-1.

Section A7.2.2. Aerobic degradation in soil (03)

Annex Point IIIA, XII 1.1

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was designed to investigate the degradation of Preventol® CMK in soil under aerobic conditions by HPLC analysis.	
5.1.1	Calculations	For Preventol® CMK a half-life of 4.4 days under aerobic conditions was evaluated graphically from data presented in Table A7_2_2_1-2 and Figure A7_2_2_1-1.	
5.2	**Results and discussion**		
5.2.1	DT_{50} value	The rate of degradation of Preventol® CMK was rapid in the duplicate soil samples. The degradation half-life determined for Preventol® CMK accounts for 4.4 days under the test conditions. For details on the degradation data it is referred to Table A7_2_2_1-2 and Figure A7_2_2_1-1. The determined half-life is in excellent agreement with Literature results of Loehr et al. (1992), who report a half life of 4.2 days under similar conditions.	X
5.2.2	Degradation products (% of a.s.)	The investigation of the degradation pathway was not subject of this study	
5.2.3	Bound residues	see 5.2.2	
5.2.4	CO_2 formation	not determined, since the study was designed to analyse primary degradation of the test substance.	
5.3	**Conclusion**	a certified standard soil was spiked at 10 mg/kg soil with a Preventol® CMK (4-Chloro-3-methylphenol) containing solution. The concentration in the soil was monitored for a test period of 19 days using a substance-specific HPLC analytical method. The test was carried out at room temperature (22°C - 23°C) in the dark under aerobic conditions. At the end of the test period of 19 days the Preventol® CMK was degraded for more than 90% (primary degradation). For Preventol® CMK a half-life of 4.4 days under aerobic conditions was calculated. The study results are in excellent agreement with Literature results of Loehr *et al.* (1992) who report a haf life of 4.2 days under similar conditions. <u>Literature:</u> Raymond C. Loehr, John E. Matthews 1(4) (1992) 339-360 Journal of Soil Contamination - Loss of organic chemicals in soil: Pure compound treatability studies.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	none	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	07/10/2011
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

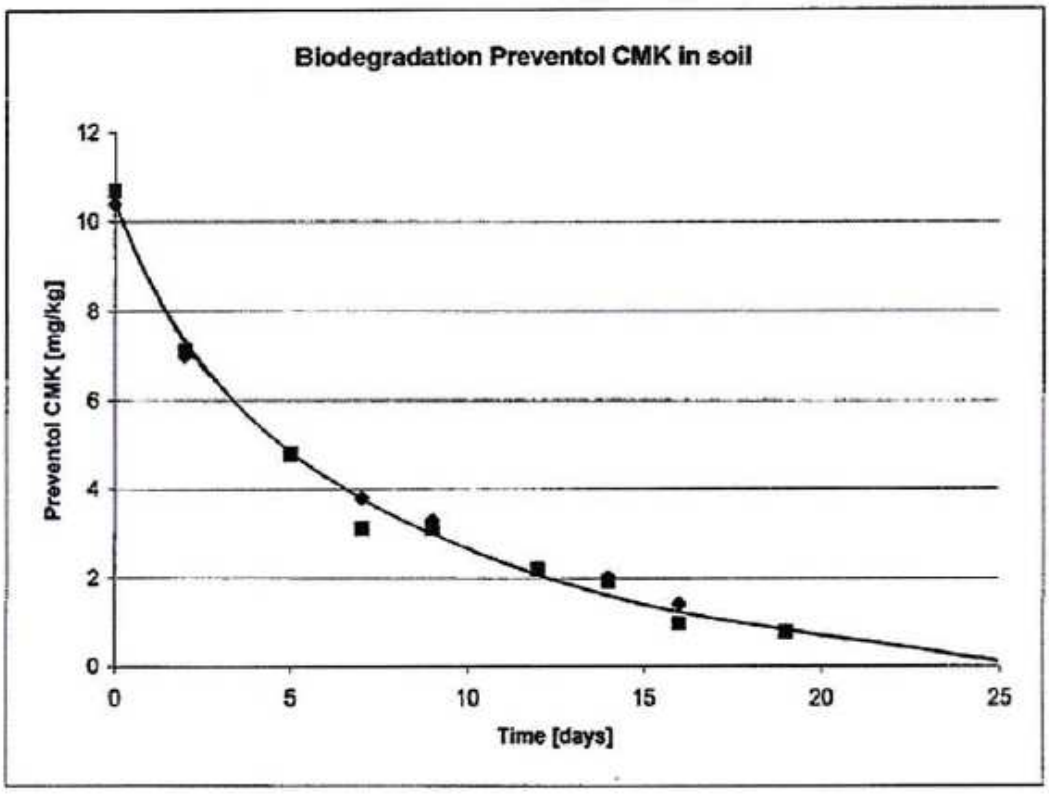
Table A7_2_2_1-1: Details on the grain size distribution and further properties of the soil

Test parameter	Unit	Test result	Test Method
pH value in $CaCl_2$	-	6.9	VDLUFA Methodbook, Vol.1, 1991, A5.1.1
Humus	%	1.9	DIN ISO 10694
Total carbon (TC)	%	1.1	DIN ISO 10694
Clay (< 0.002 mm)	%	12.9	DIN ISO 19683 – 2.April 1973
Fine silt (0.002 – 0.0063 mm)	%	5.9	DIN ISO 19683 – 2.April 1973
Medium silt (0.0063 – 0.02 mm)	%	9.7	DIN ISO 19683 – 2.April 1973
Coarse silt (0.02 – 0.063 mm)	%	17.6	DIN ISO 19683 – 2.April 1973
Fine sand (0.063 – 0.2 mm)	%	19.8	DIN ISO 19683 – 2.April 1973
Medium sand (0.2 – 0.63 mm)	%	29.3	DIN ISO 19683 – 2.April 1973
Coarse sand (0.63 – 2mm)	%	4.8	IN ISO 19683 – 2.April 1973

Table A7_2_2_1-2: The rate of degradation of Preventol® CMK in aerobic soil

Time [day]	Bottle 1 [mg CMK / kg soil]	Bottle 2 [mg CMK / kg soil]	arithmetic average [mg CMK / kg soil] (± standard deviation)
0	10.4	10.7	10.6 ± 0.21
2	7.0	7.1	7.1 ± 0.07
5	4.8	4.8	4.8 ± 0.0
7	3.8	3.1	3.5 ± 0.49
9	3.3	3.1	3.2 ± 0.14
12	2.2	2.2	2.2 ± 0.0
14	2.0	1.9	2.0 ± 0.07
16	1.4	0.96	1.18 ± 0.31
19	0.79	0.77	0.78 ± 0.01

Figure A7_2_2_1-1: Curve progression for the degradation of Preventol® CMK in aerobic soil



Section 7.2.2 **Annex Point IIIA 12.1.3**	**Fate and behaviour in soil** **Aerobic degradation in soil, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	
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	26/12/2011	
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*	

Section 7.2.2 Annex Point IIIA 12.1.3	Fate and behaviour in soil Aerobic degradation in soil, further studies
Conclusion Remarks	*Discuss if deviating from view of rapporteur member state*

Annex I : Bilateral discussions with the Applicant about the requirement of additional data on the fate of CMK in soil

Statement on the anaerobic/aerobic degradation of Chlorocresol (CMK) in soil with special emphasis on the persistency criteria and the extent of soil exposure – Update Oct. 18, 2011

In the course of the review of the dossier for the a.s. *p*-Chloro-*m*-cresol (CMK, 4-chloro-3-methylphenol, chlorocresol; CAS 59-50-7) notified for the BPD Product Types 1, 2, 3, 6, 9 and 13 we like to further expand on the request for a biodegradation study of CMK in soil.

The main argument delivered by RMS for requesting a complete study on biodegradation of CMK in soil (including quantification of bound residues and possible metabolites) is based on the need for assessing a potential persistency of the a.s. or its metabolite(s) in soil according to Annex VI of the BPD. Together with the following statement the applicant delivers additional information (several documents are submitted with this argumentation) which approves that the submission of a complete biodegradation study in soil including identification and determination of breakdown products (OECD GL 307) is not considered necessary. This conclusion is drawn since the biodegradation extent and rate constants of CMK disprove the persistency of the substance in soil and further more because exposure of soil is considered to be insignificant (due to several degradation and dissipation processes taking place beforehand the substance might reach the terrestrial compartment).

The criterion for persistency in soil according to Paragraph 85 in Annex VI of the BPD stressed by RMS defines that a substance which is not degraded in soil during field testing over a period of one year and/or a substance that forms non-extractable residues in amounts exceeding 70% of the initial dose after 100 days with a mineralization rate of less than 5% in 100 days shall not be authorised by Member States when unacceptable contamination of soil is likely to occur. With respect to the environmental fate of CMK in aerobic soil it can be doubtlessly concluded from several publicised studies on biodegradation that CMK disappears rapidly with half-life values ranging between 4.1 and 21 days for aerobic degradation of CMK in soil (Sattar 1989, Loehr & Matthews 1992). Both authors prove (primary) degradation of the test substance, but do not identify potential breakdown products and their environmental fate in soil. The results of Loehr & Matthews (1992) have recently been confirmed in an aerobic biodegradation study by Nitsche (2011) which was submitted in summer 2011 in order to be included into the BPD dossier. Nitsche reports a half-life of 4.4 days for a certified sandy silt loam soil at an application rate of approx. 10 mg chlorocresol/kg soil. In the study the

dissipation of the parent compound was analytical monitored by HPLC methodology. For the rapid dissipation at least primary degradation of the chlorocresol must be assumed.

As explained in the document "Fate and Toxicity of potential CMK metabolites in soil_27122010" the degradation pathway of CMK can be described by referring to the commonly known degradation scheme for phenolic compounds (Phenol, BUA Report 209 from May 1997, p.142). According to this degradation scheme phenolic compounds are first oxidised (by formation of catechol as a transient product) before cleavage of the aromatic ring structure occurs leading to the formation of carbonyl and carbonic acid structures (as polar substances), which are rapidly further degraded either to CO_2 or react chemically with the organic matter in the soil. For clarification on the mineralization rate of CMK and the quantification of potentially built non-extractable residues (NERs) scientific read across as established under REACH may be performed (cross-reading for biodegradation studies in soil are scientifically justified as both compounds are phenolic structures and both chemicals show a ready biodegradation in aquatic medium).

Hence, relevant results from a study by Thomas W. Federle (1988) on the mineralization of phenol, benzoic acid and benzylamine in soil followed by $^{14}CO_2$ evolution are laid down in the following. Analysis of the mineralization of aromatic compounds is performed as a function of depths in two 20-m sandy soil profiles (vadose and saturated zones). Collected soil samples from two different sites in north central Wisconsin and various depths were adjusted to 20-25% water content and spiked with 50 ng/g soil of the ^{14}C-ring-labeled compounds. The first-order rate constant for phenol mineralization was determined by non-linear regression for several soil depths and ranged between 0.31 and 3.3 d^{-1} with the average being 1.2 d^{-1} (= mean half-life of 0.56 days). Degradation rates in the saturated zone were comparable with those in the vadose zone. The degradation pathway for Phenol is oxidation to catechol with subsequent *meta* or *ortho* cleavage of the ring. Mineralization of phenol and the other two phenolic compunds occurred in all samples without a lag-period. With regard to the recovery of $^{14}CO_2$ it is shown that phenol is mineralised between 17 and 32% of applied RA during 64 days of incubation (Federle 1988). Compared with the recovery rates of benzoic acid and benzylamine (up to 53%) this reduced mineralization extend can be explained by volatilisation of the compound due to its high vapour pressure. After the incubation period of 64 days the radioactivity (RA) remaining in the test systems was fractionated. In the biotic samples (called "live samples" by the author in contrast to the abiotic controls) for phenol 91 – 95% of the residual RA was not extractable with water. In the abiotic controls (containing formalin) the non-extractable residues accounted for 57 – 29 % of the residual RA indicating that the additionally bound fraction of phenol in the live samples likely refers to the amount associated with the insoluble organic matter. Total recoveries of radioactivity from these samples were 60-72% for phenol. Figure 3 of the publication illustrates the results:

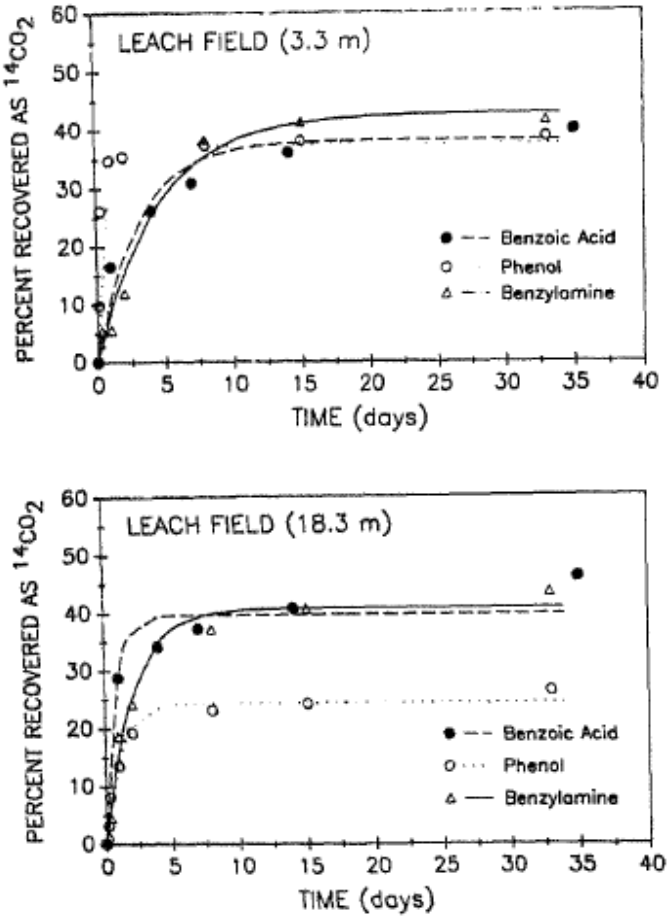


FIG. 3. Percent of radiolabeled benzoic acid, phenol, and benzylamine recovered as $^{14}CO_2$ over time in representative samples from 3.3 (top) and 18.3 m (bottom) in the leach-field profile. The curves show the fitted first-order functions.

Federle (1988) as well as Subba-Rao and Alexander (1982) state that the mineralization extend of aromatic substances becomes less when sorption/incorporation of the polar breakdown products (carbonyl and carbonic acid structures) into humic material becomes greater. From these study results the following conclusions can be drawn for the environmental fate of phenol in soil:

Degradation of phenol takes place with an average half-life of 0.56 days, whereas the ultimate degradation to $^{14}CO_2$ accounts to 17 and 32% during 64 days. An additional not quantified amount of phenol is assumed to volatise from soil, and a maximum fraction of 68.7% of AR (95.5% of AR bound to soil particles from 72% of maximum total AR recoveries in the test systems after incubation) might in a worst-case situation be bound to the soil compartment which constrains bioavailability. The discrepancy between the water-extractable portion of RA in biotic and abiotic samples indicate that breakdown products of phenol are incorporated into biomass (as humic acids) as demonstrated by Stott *et al.* (1983) for catechol being the primary breakdown product of phenol. Federle (1988) proves in his study that the analysed aromatic compounds are rapidly degraded by microbes in subsurface environments, which is properly due to preadaptation for utilization of natural aromatic substrates. Preadaption is very likely a consequence of the fact that a high proportion of humic materials consists

of aromatic moieties. Moreover, the author concludes that the metabolism pathways for the three analysed aromatic compounds can be described to follow common or parallel schemes. When finally comparing the data described above for the degradation of phenol in soil with the persistency criterion as described in Annex VI of the BPD it can be derived by cross-reading that also CMK and its breakdown products may be mineralised to more than 5% in 100 days. Therefore, the parent as well as potential breakdown products can be concluded not to fulfil the persistency criterion.

To support the ultimate degradation and mineralization of chlorocresol in soil further read-across may be taken into consideration: the scientific publications by Haider et al. (1974) and Weijnen et al. (1989) investigate the mineralisation rate (measured via $^{14}CO_2$ evolution) of phenol and/or chlorophenols under aerobic conditions in soil. The studies support the assumption that simple phenolic compounds as 4-chloro-3-methylphenol (Chlorocresol) should be mineralized for more than 5% in 100 days:

The study performed by Haider et al. (1974) on the mineralization of ^{14}C-labelled chlorinated aromatic derivates in a natural soil from Flachstöckchenheim near Braunschweig, Germany (1.26% C; 0.12% N; pH(KCl) 7,1) by soil bacteria revealed mineralisation rates ranging between 25 and 65% for mono-, di- and trichlorinated phenolic substances after 10 weeks. Most extensive degradation rates after 10 weeks were found for phenol (65% CO_2), followed by a mixture of 2,4,6/2,4,5-Trichlorphenol (51% CO_2), and a mixture of 2,4/2,6-Dichlorophenol (48% CO_2). 4-Chlorophenol as a structurally related compound to chlorocresol regarding the para-position for the chloride molecule proved CO_2 evolution up to 35%; 2-Chlorophenol showed a mineralisation extent of 25% (Haider et al. 1974, Table 3). Furthermore, preliminary degradation studies showed that soil samples from different origins revealed similar abilities for degradation of the contemplated aromatic derivates.

From Haider et al. 1974, Table 3: evolution of labelled CO_2 – data for the phenolic compounds

COMPOUND	3 Days	1 Week	2 Weeks	5 Weeks	10 Weeks
Phenol	45,5	48	52	60	65
2-Chlorphenol	7,5	13	14,7	21	25
4-Chlorphenol	15,4	22,2	24	31	35
Dichlorphenol	1,4	31,4	35	43	48
Trichlorphenol	1,6	35	38	47	51

A similar mineralization extent for 4-Chlorophenol by aerobic soil bacteria was detected by Weijnen et al. (1989) in a soil typ from The Netherlands. Their experimental research with C-labelled 4-chlorophenol in Rolde soil (3.3% org. C; pH(KCl) 5,8) showed a CO_2 evolution of 18% within 6 weeks (44 days) of study duration. The mineralisation extent for 3,4-Dichlorphenol in the same soil type accounted for 13% within 41 days. The data as presented in tables 5.8 and 5.9 of the study report is copied below:

Table 5.8: break-up of radioactive labelled 4-monochlorophenol and formation of labelled CO_2 in Rolde soil. Percentage recovery related to the quantity of added radioactivity.

		AEROOB		STERILE	
SAMPLE	TIME (DAYS)	REC-CF (%)	REC-CO_2 (%)	REC-CF (%)	REC-CO_2 (%)
1	0	83.26	0.02		0.15
2	0	77.17	0.53	98.54	0.00
3	0	90.20	0.20	95.94	0.05
4	1	63.47	1.35	95.16	0.01
5	1	72.36	0.88	92.79	
6	1	70.18	1.78	93.13	
7	3	49.43	0.18	95.63	0.00
8	3	60.30	4.04	96.77	
9	3	62.54	4.14	99.72	
10	7	39.52	9.01	94.61	0.03
11	7	4.33	19.93	95.33	
12	7	39.60	9.25	94.72	
13	10	6.47	20.47	96.09	0.03
14	10	25.41	13.86	98.53	
15	10	23.20	7.50	95.78	
16	14	3.74	23.30	95.17	0.01
17	14	4.59	20.90	97.31	
18	14	3.51	22.51	93.13	
19	21	3.27	21.86	99.04	0.03
20	21	4.82	16.31	90.77	
21	21	2.87		100.24	
22	44	4.48	17.42	89.85	0.02
23	44	4.49	17.59	92.65	0.02

Table 5.9: break-up of radioactive labelled 3,4-dichlorophenol and formation of labelled CO_2 in Rolde soil. Percentage recovery related to the quantity of added radioactivity.

		AEROOB		STERILE	
SAMPLE	TIME (DAYS)	REC-CF (%)	REC-CO_2 (%)	REC-CF (%)	REC-CO_2 (%)
1	0	87.27	0.03		0.01
2	0	89.32	0.03	95.37	0.01
3	0	86.44	0.03	95.38	0.01
4	3	82.71	0.95	93.66	0.00
5	3	83.75	0.92	93.79	
6	3	81.93	0.88	91.74	
7	7	71.79	2.00	91.44	0.01
8	7	75.15	1.95	93.08	
9	7	70.59	1.30	92.72	
10	14	39.74	4.51	92.68	0.01
11	14	49.92	3.75	91.84	
12	14	34.46	2.65	90.78	
13	22	22.81	9.31	97.04	0.01
14	22	21.17	9.88	91.96	
15	22	29.91	8.40	92.86	
16	27	18.10		83.36	0.01
17	27	15.07	11.08	80.49	
18	27	15.66	10.79	80.72	
19	41	11.22			0.02
20	41	10.63	12.45	94.68	
21	41	11.56	13.91	93.46	

The kinetic aspects of aerobic biodegradation of anthropogenic organic compounds by subsurface microbes were investigated by Swindoll *et al.* (1988). By means of radiotracer methods the biodegradation and the $^{14}CO_2$ mineralization of six natural and nine xenobiotic organic compounds, *inter alia* m-cresol, was studied in a slurry experiment using a Oklahoma subsurface sand soil taken from the aquifer at 4.5 to 5.5 m depth. The data was evaluated with both a first-order and a Michaelis-Menten approach. Kinetic data for phenolic substances reveal reasonable turnover times T(n) for $^{14}CO_2$ formation and complete dissipation of the substance. The turnover times are calculated for a normalized concentration of 0.5µg/g; T(n) for Phenol is reported to be 805.9 h, for m-Cresol 1370.3 hours is found showing a rapid dissipation and mineralization. In the relevant tables 2 and 3 only kinetic evaluation parameters are given, no further measured values are provided in the publication.

From Swindell et al. 1988, Table 2 – Data for Phenol:

Table 2. Calculated turnover times (T_n), first-order rate constants (K_1), maximum velocity (V_{max}) and half-saturation constants (K') for natural substrates

Compound	Soil	T_n (h)	Metabolic parameter	K_1 (h^{-1}; $\times 10^{-3}$)	SE ($\times 10^{-3}$)	V_{max} (ng/g/h)	SE	K' (ng/g; $\times 10^4$)	SE ($\times 10^4$)
Phenol	9S2	805.9	Respiration	3.45	0.41	10.10	1.30	5.00	0.00
			Uptake	55.70	7.70	NS	NS	NS	NS
			Total	58.10	7.80	NS	NS	NS	NS

SE, standard error of estimate; NS, not saturated.

From Swindell et al. 1988, Table 3 – Data for m-Cresol:

Table 3. Calculated turnover times (T_n), first-order rate constants (K_1), maximum velocity (V_{max}) and half-saturation constants (K') for xenobiotic compounds

Compound	Soil	T_n (h^{-1})	Metabolic parameter	K_1 (h^{-1}; $\times 10^{-5}$)	SE ($\times 10^{-5}$)	V_{max} (ng/g/h)	SE	K' (ng/g; $\times 10^4$)	SE ($\times 10^4$)
m-Cresol	9S2	1,370.3	Respiration	51.10	2.90	NS	NS	NS	NS
			Uptake	23.40	4.90	NS	NS	NS	NS
			Total	74.50	5.30	NS	NS	NS	NS

SE, standard error of estimate; NS, not saturated.

Another strong argumentation for non-submission of a new and extended study on aerobic degradation in soil using radioactive labelled material is that CMK is proven in three further experiments to disappear effectively by biotic and abiotic processes so that only negligible amounts of the parent could reach either a sewage treatment plant (STP) and subsequently enter the soil compartment *via* sludge application on agricultural land (no direct emissions to soil are expected due to the product types applied for in the dossiers submitted in 2007), or via direct irrigation of stable cleaning waters on agricultural land:

> Vaporization Experiment

The abiotic dissipation of Chlorocresol from surfaces is proven to be highly effective due to volatilisation processes. Hence, after a certain time period only negligible residues of the active substance are left on disinfected surfaces. The applicant conducted a study on the vaporisation behaviour of 4-chloro-3-methylphenol within the product Neopredisan® 135-1 (applied as PT3 product). This study (Gerharz 2011c) which is submitted with this document, shows that CMK evaporates consistently from an inert surface leaving less than 1% of the originally applied amount after 96 hours. When considering the negligible CMK-load to be expected in STP together with the fact that elimination of CMK in a STP is proven by monitoring data to be highly effective (up to 99.9% removal) the potential exposure of soil to CMK residues via sludge is maximum of insignificant extent if not zero. Cleaning waters from disinfected stables which might be applied directly on agricultural land is also not likely to contain CMK as the substance has evaporated from the surfaces during the normal cleaning intervals of several weeks. Thus, the suitability of the phrase

"unacceptable contamination of soil" which is the condition for the relevance of the persistency criteria in Annex VI is not given.

> Degradation in Stable Cleaning Waters

Apart from monitoring data on the effective biodegradation of the a.s. during waste waster treatment revealing elimination rates of up to 99.9% (*cf.* Section 3.3.3.1 in Doc.II-B), it is demonstrated by monitoring data that in a washing water sample resulting from stable spouting of a previously disinfected (laying hen) stable no CMK was present (Gerharz 2011a). The disinfection event (wetting the surface and waiting approx. 12-24 h until dry) took place before new animals were introduced into the stable, i.e. approx. 12 months before the next cleaning step with water was carried out.

Furthermore, it is experimentally shown by the applicant that CMK newly introduced into a stable cleaning water (received from stable spouting of a previously disinfected (laying hen) stable) is rapidly biodegraded in the washing water sample. As described in the Test Report on CMK degradation in a liquid environment (Gerharz 2011a) submitted with this document, no traces of the compound could be detected in the washing water after the cleaning event although the stable was treated with a chlorocresol-containing product previously. So the washing water was spiked with two different concentrations of CMK (5 mg/kg and 10 mg/kg) and the subsequent degradation was monitored by HPLC analysis of the duplicate samples. For both test concentrations a nearly complete degradation reaching less than 1% of the applied amount was determined for CMK in the test period of 8 days. The substance degraded with a half-life of 2-3 days.

> Degradation in Manure

As described in the ESD for PT18 (OECD 2006) and PT3 (EC 2010) potential residues of the a.s. enter the slurry/manure container for storage before application to agricultural land is expected. Thus, further reduction of the CMK content in slurry/manure for PT3 products is possible due to biological degradation in the storage containers. Despite the fact that no official guideline is available for analysis of this degradation pathway the applicant conducted a degradation study by analysing CMK degradation in pork liquid manure under anaerobic conditions. The corresponding Test Report (Gerharz 2011b) is attached to this document. For the test a fresh manure sample received from ChemCon GmbH was submitted to HPLC analysis for determination of possible CMK residues, which could not be detected. Subsequently, the sample was spiked with a CMK-containing solution at a concentration of 3 mg/kg CMK under argon air flow to maintain anaerobic conditions. The sampling schedule for duplicate HPLC analysis of the CMK concentration in manure was at day 0, day 20, day 27 and day 34. After the test period of 34 days the 4-chloro-3-cresol concentration was less than 17% of applied CMK, i.e. Chlorocresol degraded with a half-life of 15 days. Sodium benzoate was used as a control substance in the test at a concentration of 100 mg/kg and revealed an experimental half-life of 3 days for degradation under anaerobic conditions. The test results for Chlorocresol prove that anaerobic storage conditions contribute to a decrease of CMK in liquid manure.

> Necessity of Soil Dissipation Studies for Readily Biodegradable Substances

Besides the fact that in literature CMK is consistently referred to as a biodegradable substance in soil with fast dissipation rates, it should be stated here that the Technical Meeting (TM III-06) hold from the 16th to the 19th of October 2006 decided to justify non-submission of a biodegradation study in soil due to the ready biodegradability of the corresponding substance in water. According to the submitted OECD tests for CMK (Müller 1992; Weyers 2007; Hanstveit & Pullens 1993) it is classified as readily biodegradable under stringent test conditions (implying a high inoculum activity and/or the presence of appropriate inoculum species being able to degrade CMK). Nevertheless, monitoring data and the already submitted water/sediment-study reveal the fast and exhaustive degradation of CMK in aqueous media.

Furthermore, a publication by Struijs et al. (1995) delivers sound arguments on expected soil biodegradation for substances which are readily biodegradable in the aquatic environment. The rational is based on the organism population density which is much higher for soil than in the OECD tests which are used to assess ready aquatic biodegradation.

Summary

Summarizing the presented facts for the active substance CMK the applicant wants to stress that there are reasonable arguments for a scientific-based waiving of a requested radioactive labelled study on biodegradation in soil. The arguments include valid and confirmed data on the fast biodegradation of the a.s. in soil, and cross-reading of results for the biodegradation rate and mineralization extent of (chloro-)phenolic compounds -including m-cresol- in soil: for chlorocresol dissipation in soil is poven to be rapid (half-life approx. 4-5 days) and mineralization is derived to be significantly above 5% in 100 days even if there were bound residues exceeding 70%. Thus, the applicant considers the aspect of persistency and environmental relevance in soil to be extensively answered and disproved.

The second explanation for non-submission of the requested soil degradation study is based on an exposure-driven argumentation proving that the use of CMK in most of the product-types will not lead to any noteworthy environmental exposure of soil to CMK and its transient breakdown products. Since the a.s. reveals a fast and effective biotic and abiotic degradation or dissipation behaviour in several media, most of them are to be passed before CMK enters the terrestrial compartment via e.g. sludge application.

As a third argument it should be taken into consideration that for a wood preservative the Technical Meeting TM III-06 decided in October 2006 that the non-submission of a biodegradation study in soil is justified if for the substance the ready biodegradability in water is shown. Scientific background for such a decision is provided.

Overall, for chlorocresol the request for a new radioactive labelled biodegradation study in soil including identification and determination of breakdown products (OECD GL 307) is considered disproportional for the applicant as it would add only insignificant scientific value for the environmental evaluation of the substance.

Literature:

Federle, T.W. (1988): Mineralization of monosubstituted aromatic compounds in unsaturated and saturated subsurface soils. Can. J. Microbiol. 34: 1037-1042.

Gerharz, T. (2011a): Test Report: Degradation of 4-chloro-3-cresol in a liquid environment (washing water after stable cleaning – stable with laying hens). Lanxess Deutschland GmbH.

Gerharz, T. (2011b): Test Report: Degradation of 4-chloro-3-cresol in pork liquid manure under anaerobic conditions. Lab Report ID: D 2011-10, Lanxess Deutschland GmbH.

Gerharz, T. (2011c): Test Report: Neopredisan 135-1. Vaporisation behaviour of 4-chloro-3-methylphenol from an inert surface (glass petri dish). Lab Report ID: D 2011-22.1.5, Lanxess Deutschland GmbH.

Haider K., Jagnow G., Kohnen R. & Lim S.U. (1974): Abbau chlorierter Benzole, Phenole und Cyclohexan-Derivate durch Benzol und Phenol verwertende Bodenbakterien unter aeroben Bedingungen. In: Arch. Microbiol. 96, 183-200.

Loehr, R.C. & Matthews, J.E. (1992): Loss of organic chemicals in soil. Pure compound treatability studies, Journal of Soil Contamination 1(4), 339-360 (already submitted: A7.2.1(02)).

Nitsche, M. (2011): Biodegradation of Preventol® CMK (4-chloro-3-methylphenol) in soil under aerobic conditions – Test Report, Lanxess Deutschland GmbH, Leverkusen, Germany. Date: 25th July, 2011

Sattar, M.A. (1989): Fate of chlorinated cresols from environmental samples, Chemosphere 19 (8/9), 1421 – 1426 (already submitted: A7.2.1(01)).

Stott, D.E., Martin, J.P., Focht, D.D., and Haider, K (1983): Biodegradation, stabilization in humus and incorporation into biomass of 2,4-D and chlorocatechol carbons. Soil Sci. Soc. Am. J.45: 66-70.

Struijs, J., and van den Berg, R. (1995): Standardized Biodegradability Tests: Extrapolation to Aerobic Environments. In: Wat. Res. 29, 255-262

Subba-Rao, R.V. and Alexander, M. (1982): Effect of sorption on mineralization of low concentrations of aromatic compounds in lake-water sample. Appl. Environ. Microbiol. 44: 659-668.

Swindoll C.M., Aelion C.M., Dobbins D.C., Jiang O., Long S.C. & Pfaender, F.K. (1988): Aerobic biodegradation of natural and xenobiotic organic compounds by subsurface microbial communities. In: Environmental Toxicology and Chemistry, Vol.7, 291-299.

Weijnen P.H.C., v.d.Berg R., v.d. Berg S. (1989): Biodegradatie van chloorfenolen in de bodem. Rapport nr. 728603005, Rijksinstituut voor Volksgezondheid en Milieuhygiene Bilthoven, NL.

Section A7.2.3.1

Annex Point IIIA, XII 1.2

Adsorption and desorption in at least three soil types and, where relevant, adsorption and desorption of metabolites and degradation products (01)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Meinerling, M. (2007): Determination of the Adsorption / Desorption behaviour of 4-Chloro-3-methylphenol (Preventol CMK) Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany, Report No. 32323195, unpublished, Date: 2007-06-20	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guidelines for Testing of Chemicals, Guideline No. 106, ”Adsorption / Desorption”; adopted January 21, 2000. Commission Directive 2001/59/EC, Method C.18, Adorption/Desorption using a batch equilibrium method (EEC Publication No. L 225, 2001).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No relevant deviations	
		3 MATERIALS AND METHODS	
3.1	**Test material**	4-Chloro-3-methylphenol (Preventol CMK)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Non-radiolabelled test substance	
3.1.3	Purity	[redacted]	

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Adsorption and desorption in at least three soil types and, where relevant, adsorption and desorption of metabolites and degradation products (01)

3.1.4	Further relevant properties	None
3.1.5	Method of analysis	Preparation of the Stock Solution: Approximately 100 mg or 50 mg of the test item were dissolved in methanol and were filled up to 100 mL or 50 mL using a volumetric flask. Standard Solutions: Appropriate volumes of the stock solution were diluted with 0.01 M $CaCl_2$ solution to obtain standard solutions in concentration range of 0.5 to 12.5 mg test item/L. HPLC-System: LaChrom, Merck Hitachi Detector: UV detector / Diode Array Detector at 280 nm Column: RP 18 (250 *4 mm) Oven Temperature: 25 °C Mobile Phase: Acetonitrile / pure water gradient mode Flow Rate: 1.0 mL/min Injection Volume: 50 – 100 µL
3.2	**Degradation products**	Degradation products tested: No
3.2.1	Method of analysis for degradation products	Not relevant (*cf.* Point 3.2)
3.3	**Reference substance**	None
3.3.1	Method of analysis for reference substance	Not relevant (*cf.* Point 3.2)
3.4	**Soil types**	Available data are given in Table A7_2_3_1-1
3.5	**Testing procedure**	
3.5.1	Test system	Four different soils, which varied in clay content, organic carbon content and pH were used (*cf.* Point 3.4). The test vessels were made of glass and did adsorb negligible amounts of Preventol CMK.
3.5.2	Test solution and Test conditions	Aqueous solvent: 0.01 M $CaCl_2$ solution was used as the aqueous solvent phase. Pure water was used to prepare the $CaCl_2$ solution. Test conditions: Air conditioned room, in the dark, 20 °C ± 2 °C Further information is given under Point 3.6.
3.6	**Test performance**	
3.6.1	Preliminary test	In a preliminary study the soil/solution ratio was estimated. The equilibrium time for adsorption and the amount of p-chloro-m-cresol adsorbed at equilibrium as well as potential adsorption of the test item on surfaces of the test vessels and the stability of the test item were

Section A7.2.3.1

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Adsorption and desorption in at least three soil types and, where relevant, adsorption and desorption of metabolites and degradation products (01)

		estimated. Conclusive information is given in Table A7_2_3_1-2.	
3.6.2	Screening test: Adsorption	In the screening test the adsorption at four different soil types was studied by means of the adsorption kinetic at a single concentration and the determination of the distribution coefficient K_d and K_{oc}. Conclusive information is given in Table A7_2_3_1-3.	
3.6.3	Screening test: Desorption	The desorption kinetics were performed to investigate whether the test item was reversibly or irreversibly adsorbed on the soil. The desorption was estimated at one concentration of the test item using 4 different soil types. Conclusive information is given in Table A7_2_3_1-4.	
3.6.4	HPLC-method	See Point 3.1.5	
3.6.5	Other test	In the test for determination of the adsorption isotherm the influence of the concentration on the extent of adsorption was investigated. Conclusive information is given in Table A7_2_3_1-5. The Freundlich desorption isotherm was determined on the soils used in the adsorption isotherms experiment. Conclusive information is given in Table A7_2_3_1-6.	
		4 RESULTS	
4.1	**Preliminary test**	The preliminary test was performed using two soil types and three different soil/solution ratios. A soil/solution ratio of 1:25 and a concentration of 100 mg test item/L in the aqueous solution resulted in an adsorption of < 80% and > 17% after 24 hours equilibrium time. Thus a soil/solution ratio of 1:25 was chosen for the screening test. After 24h the adsorption did still increase. Therefore an equilibrium time of 48h was considered for the screening test. p-Chlor-m-cresol was stable under the test conditions and did not adsorb on the surface of the test vessel. From analysis of the control soils no interfering compounds were detected. 28 and 96% of the nominal test item amount could be analytically recovered after adsorption.	X
4.2	**Screening test: Adsorption**	In the screening test the adsorption kinetics were estimated at one concentration of the test item using four different soil types. The results are provided in Table A7_2_3_1-7. The equilibrium was reached after 48h incubation time (change in adsorption was < 5%). The calculated distribution coefficient was in the range of 1.9 to 11.8 mL/g. The corresponding adsorption coefficient was in range of 161 to 508 mL/g.	
4.3	**Screening test: Desorption**	The desorption kinetics were performed to investigate whether the test item was reversibly or irreversibly adsorbed on the soil. The desorption was estimated at one concentration of the test item using 4 different soil types. The desorption of adsorbed test item from the different soil types ranged from neglible desorption up to 22% desorption.	X
4.4	**Calculations**	The Freundlich adsorption isotherms equation relates the amount of test item adsorbed on soil to the concentration of test item in solution at equilibrium. The equations in linear form were calculated.	

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Annex Point IIIA, XII 1.2

Adsorption and desorption in at least three soil types and, where relevant, adsorption and desorption of metabolites and degradation products (01)

4.4.1	Ka , Kd	The adsorption coefficients Ka (adsorption) and Kd (desorption) were derived from the screening test and the adsorption isotherm according to Freundlich. They are summarized in Tables A7_2_3_1-7 to A7_2_3_1-9	
4.4.2	Ka_{oc} , Kd_{oc}	The Ka_{oc} coefficients calculated from the screening test and the Freundlich adorption isotherms are presented in Tables A7_2_3_1-7 and A7_2_3_1-8	
4.5	**Degradation product(s)**	No item for investigation.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Aqueous solutions of p-chloro-m-cresol were equilibrated with soil and the adsorption and desorption coefficients and constants were determined. The concentration of the test item was determined using HPLC method. The test system and test performance is described in Points 3.5 and 3.6 (batch equilibrium procedure). The experiment was conducted according to OECD Guidelines for Testing of Chemicals, Guideline No. 106 and Commission Directive 2001/59/EC, Method C.18. No relevant deviations from the guidelines occurred.	
5.2	**Results and discussion**	From results of the preliminary test the soil/solution ratio of 1:25 was chosen in an attempt to fit the Freundlich model. In the screening test the adsorption kinetics were estimated at one concentration of the test item using four soil types. The calculated distribution coefficients were in the range of 1.9 to 11.8 mL/g. The corresponding adsorption coefficients related to organic carbon were in the range from 160.9 to 508.2 mL/g. The Freundlich adsorption coefficients (K_F values) varied between 3 and 18 [$\mu g^{1-1n}(cm^3)^{1/n}g^{-1}$]. Related to organic carbon, adsoption coefficients between 270 and 981 mL/g had been calculated. The slope of the isotherm is below 0.75 for all four soils. Freundlich coefficients determined for the desorption were lower as those determind for the adsorption, i.e. 0.5 to 1.7, indicating that the compound once adsorbed is transferred back to the soil pore water. Conclusive information is given in Tables A7_2_3_1-7 to A7_2_3_1-9.	X
5.2.1	Adsorbed a.s. [%]	In the screening test the percent adsorbed varied between 42.4% and 77.9% (*cf.* Table A7_2_3_1-7).	
5.2.2	K_a	Screening test: 1.9, 7.6, 9.3 and 11.8 mL/g Adsorption isotherm: 3, 11, 14 and 18 [$\mu g^{1-1n}(cm^3)^{1/n}g^{-1}$]	
5.2.3	K_d	Adsorption isotherm: 0.5, 1.2, 1.5, 1.7 [$\mu g^{1-1n}(cm^3)^{1/n}g^{-1}$]	X
5.2.4	Ka_{oc}	Screening test: 160.9, 230.3, 497.9, 508.2 mL/g Adsorption isotherm: 270, 322, 598, 981 mL7g	
5.2.5	Ka/Kd	Adsorption isotherm: 2.5, 9.3, 10.6, 22.0	
5.2.6	Degradation products (% of a.s.)	Not relevant (*cf.* Point 3.2)	

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Adsorption and desorption in at least three soil types and, where relevant, adsorption and desorption of metabolites and degradation products (01)

5.3	**Conclusion**	Based on the classification system according to Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973), compounds having K_{oc} values within the range of 130-690 are considered to be of low mobility and those having K_{oc} values > 690 are considered to be immobile. Taking into consideration the K_{oc} values obtained in this study, p-chloro-m-cresol has to be evaluated as low mobile or immobile in soils.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	November 2008
Materials and Methods	[redacted]

Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

Remarks	[redacted]
	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 A7_2_3_1-1: Classification and physico-chemical properties of soils used as adsorbents X

	Soil A	**Soil B**	**Soil C**	**Soil D**
Classification	Loamy sand	Sand soil	Clayey loam soil	Eurosoil 3
Origin	LUFA Speyer, Germany	LUFA Speyer, Germany	LUFA Speyer, Germany	IRMM Geel
Sand [%]	n.r.	n.r.	n.r.	n.r.
Silt [%]	n.r.	n.r.	n.r.	n.r.
Clay [%]	14.9 ± 2.1	8.4 ± 0.7	65.2 ± 1.8	17.0
Organic carbon [%]	2.36 ± 0.22	1.15 ± 0.29	~~1.15 ± 0.29~~ **1.83 ± 0.32**	3.3
pH ($CaCl_2$)	5.6 ± 0.4	6.0 ± 0.7	6.8 ± 0.3	5.9
Cation exchange capacity (meq/100 g)	n.r.	n.r.	n.r.	n.r.

n.r. = not reported

Table A7_2_3_1-2: Test system and test conditions of the preliminary test X

Test System:	Soils A and C were used (*cf.* Table A7_2_3_1-1).
Soil / Solution Ratio:	Three soil to solution ratios were used. The ratios were 1:1, 1:5 and 1:25. 2g, 10 g and 50 g of the soils in combination with 50 mL aqueous solution were used.
Test Item Solution:	Approximately 100 mg of the test item were dissolved in methanol and were filled up to 100 mL using a volumetric flask. The stock solution was diluted with 0.01 M $CaCl_2$ solution to a get a concentration of 100 mg/L. The concentration of the test item in 0.01 M $CaCl_2$ solution was below the water solubility of the test item.
Preparation of the Test Soils:	The air dried soil samples were equilibrated by shaking with 45 mL of 0.01 M $CaCl_2$ solution overnight. Afterwards 5 mL of the diluted test item solution (100 mg/L) were added in order to adjust the final volume to 50 mL.
Performance of the Test:	The mixtures were shaken. After a period of 4, 24 and 48h the contact time was finished and samples were collected. Therefore, the suspensions were centrifuged and filtered. The aqueous solutions were analysed.
Control Sample:	Control samples with only the test item solution (without soil) were subjected to precisely the same steps as the test system, in order to check the stability of the test item in $CaCl_2$ solution and its possible adsorption on the surface.
Blank Sample:	Blank run per soil with the maximum amount of soil and total volume of $CaCl_2$ solution were subjected to the same test procedure. This served as a background control during the analysis to detect interfering compounds or contaminated soils.
Replicates:	Two replicates per each test soil/volume ratio and two replicates per control and blank sample were run.
Analysis of Aqueous Solutions:	The concentration of the test item in the aqueous phase was determined using HPLC method.
Analysis of Soil Samples:	~~The soil of the 48h samples (48h incubation time) were analysed for the test item. Thus, the aqueous phase was recovered as much as possible and 0.01 M $CaCl_2$ solution as solvent was added to the soil. Two successive extractions steps were performed. The extracts were analysed using HPLC method.~~ **No analysis of soil samples were carried out during the preliminary test. The amount of test substance adsorbed on the soil sample was calculated as the difference between the amount of test substance initially present in solution and the amount remaining at the end of the experiment (indirect method).**
pH Value:	The pH of the aqueous phase was measured before and after contact of the test item with the soil.

Table A7_2_3_1-3: Test system and test conditions of the screening adsorption test X

Test System:	Four soils were used (*cf.* Table A7_2_3_1-1).
Soil / Solution Ratio:	10 g of the soils in combination with 25 mL aqueous solution corresponding to a soil/solution ratio of 1:2.5 were used.
Test Item Solution:	Approximately 50 mg of the test item were dissolved in methanol and it was filled up to 50 mL using a volumetric flask. For performance of the test the stock solution was diluted to 100 mg/L in 0.01 M $CaCl_2$ solution. The concentration of the test item in 0.01 M $CaCl_2$ solution was below the water solubility of the test item.
Preparation of the Test Soils:	The air dried soil samples were equilibrated by shaking with 22.5 mL of 0.01 M $CaCl_2$ solution overnight. Afterwards 2.5 mL of the diluted test item solution (100 mg/L) were added in order to adjust the final volume to 25 mL. The volume of the added stock solution did not exceed 10% of the final 25 mL volume.
Performance of the Test:	The mixtures were shaken. After a period of 8h, 24h and 48h the contact time was finished and samples were collected. Therefore, the suspensions were centrifuged and filtered to obtain a clear solution. The aqueous solutions were analysed.
Control Sample:	Control samples with only the test item solution (without soil) were subjected to precisely the same steps as the test system, in order to check the stability of the test item in $CaCl_2$ solution and its possible adsorption on the surface.
Blank Sample:	Blank run per soil with the maximum amount of soil and total volume of $CaCl_2$ solution were subjected to the same test procedure. This served as a background control during the analysis to detect interfering compounds or contaminated soils.
Replicates:	Two replicates per each test soil/volume ratio and one replicate per control and blank sample were run.
Analysis of Aqueous Solutions:	The concentration of the test item in the aqueous phase was determined using HPLC method.
Analysis of Soil Samples:	The soil of the 48h samples (48h incubation time) were analysed for the test item. Thus, the aqueous phase was recovered as much as possible and a **first extraction was performed with $CaCl_2$ for one hour. A following extraction was carried out with a** solvent mixture of 50% acetonitrile / 50% pure water **for one hour.** ~~was added to the soil. Two successive extractions steps were performed.~~ The extracts were analysed using HPLC method.
pH Value:	The pH of the aqueous phase was measured before and after contact of the test item with the soil.

Table A7_2_3_1-4: Test system and test conditions of the screening desorption test

Test System:	Four test soils were used (*cf.* Table A7_2_3_1-1).
Soil / Solution Ratio:	10 g of the soil in combination with 25 mL aqueous phase was used.
Test Item Solution:	Approximately 50 mg of the test item were dissolved in methanol and it was filled up to 50 mL using a volumetric flask. For performance of the test the stock solution was diluted to 100 mg/L in 0.01 M $CaCl_2$ solution.
Preparation of the Test Soils:	The air dried soil samples were equilibrated by shaking with 22.5 mL of 0.01 M $CaCl_2$ solution overnight. Afterwards 2.5 mL of the stock solution of the test item was added in order to adjust the final volume.
Performance of the Test:	The mixtures were shaken until adsorption equilibrium was reached (the same time interval as in the adsorption kinetic experiment). After this time the phases were separated by centrifugation. The aqueous phase was removed as much as possible. The volume of solution removed was replaced by an equal volume of 0.01 M $CaCl_2$. The new mixtures were shaken again. After defined time intervals (8h, 24h and 48h) the suspension was filtered. The aqueous phases were recovered and were analysed.
Replicates:	In case of each experiment (one soil and one test item concentration) 6 samples were prepared allowing duplicate sampling after the specified time intervals.
Control Sample:	A control sample with only the test item solution (without soil) was subjected to precisely the same steps as the test system.
Blank Sample:	One blank run per soil with the same amount of soil and total volume of $CaCl_2$ solution was subjected to the same test procedure. This solution served as a background control during the analysis to detect interfering compounds or contaminated soils.
Analysis of Aqueous Solutions:	The concentration of the test item in the aqueous phase was determined using HPLC method.
pH Value:	The pH of the aqueous phase was measured before and after contact with the soil.

Table A7_2_3_1-5: Test system and conditions of the Freundlich adsorption test X

Test System:	Four test soils were used ~~(see 62).~~
Soil / Solution Ratio:	10 g of the soil and 25 mL aqueous phase were used.
Test Item Solution:	Approximately 500 mg of the test item were dissolved in methanol and it was filled up to 50 mL using a volumetric flask. For performance of the test the stock solution was diluted to 100 mg/L, 250 mg/L, 500 mg/L and 750 mg/L in 0.01 M $CaCl_2$ solution. The concentrations did cover two orders of magnitude. The test item was dissolved in methanol.
Preparation of the Test Soils:	The air dried soil samples were equilibrated by shaking with 22.5 mL of 0.01 M $CaCl_2$ solution overnight. Afterwards 2.5 mL of the solution of the test item were added in order to adjust the final volume. The volume of the added stock solution did not exceed 10% of the final volume.
Performance of the Test:	The mixtures were shaken until equilibrium was reached. After 48h samples were collected. The suspension was centrifuged and filtered to obtain a clear solution. The aqueous solutions were analysed.
Replicates:	Each experiment (one soil and one test item concentration) was done at least in duplicate.
Control Sample:	A control sample with only the test item solution (without soil) was subjected to precisely the same steps as the test system.
Blank Sample:	One blank run per soil with the same amount of soil and total volume of $CaCl_2$ solution were subjected to the same test procedure. The blank sample served as a background control during the analysis to detect interfering compounds or contaminated soils.
Analysis of Aqueous Solutions:	At the end of the adsorption step the concentration of the test item in the aqueous phase was determined using HPLC method.
pH Value:	The pH of the aqueous phase was measured before and after contact with the soil.

Table A7_2_3_1-6: Test system and conditions of the Freundlich desorption test

Test System:	The soils loaded in the adsorption isotherms experiment were used. In the adsorption step the soils were loaded with test item (*cf.* Table A7_2_3_1-5).
Preparation of the Test Soils:	As described in Table A7_2_3_1-5.
Performance of the Test:	After end of the adsorption step the supernatant was removed from the soil by decanting. The volume of supernatant was measured and replaced by an equal volume of 0.01 M $CaCl_2$. The new mixtures was shaken again until desorption equilibrium was reached. Afterwards the aqueous phases were recovered and were analysed.
Replicates:	Each experiment (one soil and one test item concentration) was done at least in duplicate.
Control Sample:	A control sample with only the test item solution (without soil) was subjected to precisely the same steps as the test system.
Blank Sample:	One blank run per soil with the same amount of soil and total volume of $CaCl_2$ solution was subjected to the same test procedure serving as a background control during the analysis to detect interfering compounds or contaminated soils.
Analysis of Aqueous Solutions:	The concentration of the test item in the aqueous phase was determined using HPLC method.
pH Value:	The pH of the aqueous phase was measured before and after contact with the soil.

Table A7_2_3_1-7: Results of the screening experiment on adsorption

soil description	% org C	time to reach ads. equilibrium [h]	adsorption [%]	distribution coefficient K_d [mL/g]	adsorption coefficient K_{oc} [mL/g]
Lufa 2.1- sand	1.2	48	42.4	1.9	160.9
Lufa 2.2 - loamy sand	2.4	48	76.5	11.8	497.9
Lufa 6S - clay	1.8	48	77.9	9.3	508.2
Eurosoil 3 - loam	3.3	48	75.0	7.6	230.3

mean value calculated from exact raw data

Table A7_2_3_1-8: Freundlich constants describing the adsorption of p-chloro-m-cresol

soil description	% org C	intercept of isotherm log K_F	slope of isotherm	Freundlich adsorption coefficient K_F^{ads} [$\mu g^{1-1n}(cm^3)^{1/n}g^{-1}$]	regression coefficient r^2	adsorption coefficient [mL/g] K_Foc
Lufa 2.1	1.2	0.51	0.611	3	0.998	270
Lufa 2.2	2.4	1.16	0.596	14	0.999	598
Lufa 6S	1.8	1.25	0.448	18	0.979	981
Eurosoil 3	3.3	1.03	0.747	11	0.999	322

* based on Freundlich adsorption equation in linear form
$\log c_s = \log K_F + 1/n * \log c_{aq}$

Table A7_2_3_1-9: Freundlich constants describing the desorption of p-chloro-m-cresol

soil description	intercept of isotherm log K_F	slope of isotherm	Freundlich desorption coefficient K_F^{des} [$\mu g^{1-1n}(cm^3)^{1/n}g^{-1}$]	regression coefficient r^2
Lufa 2.1	0.08	0.659	1.2	0.958
Lufa 2.2	0.16	0.996	1.5	0.999
Lufa 6S	0.24	0.967	1.7	1.000
Eurosoil3	-0.31	1.192	0.5	0.992

* based on Freundlich desorption equation in linear form
$\log c_s = \log K_F + 1/n * \log c_{aq}$

Table A7_2_3_1-10: Mass balance of the screening test after 2 extraction with $CaCl_2$-solution for all soil types

	Mass nominal in solution [µg]	Mass measured in solution [µg]	1st extraction – Mass found [µg]	2nd extraction – Mass found [µg]	Total mass adsorbed on soil [µg]	Mass balance [%]
Lufa 2.1	253.7	145.7	18.0	31.6	49.6	77
Lufa 2.1	253.4	148.5	17.9	28.5	46.4	76.9
Lufa 2.2	253.8	51.2	0.3	24.9	25.2	30.1
Lufa 2.2	253.7	49.5	-1.0	23.8	22.8	28.5
Lufa 6S	254.0	35.6	-9.3	16.1	6.8	16.7
Lufa 6S	253.5	41.2	4.8	35.6	40.4	32.2
Eurosoil 3	253.5	57.4	28.6	152.8	181.4	94.2
Eurosoil 3	253.4	53.0	24.7	181.3	206.0	102.2

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1 REFERENCE

1.1	**Reference**	Meinerling, M. (2008): Determination of the Stability of 4-Chloro-3-methylphenol (Preventol CMK) in Soils of an Adsorption/Desorption Study. IBACON, GmbH, Rossdorf, Germany, Report No. 45821195, Date: 2008-11-17.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD test guideline 106 (“Adsorption / Desorption”), adopted 2001, Equals to Commission Directive 2001/59/EC, Method C.18 (2001)
2.2	**GLP**	Yes
2.3	**Deviations**	Refer to attached Figure A7.1.3_3 for detailed description of deviations from study plan (page 8 of this study summary).

3 MATERIALS AND METHODS

3.1	**Test material**	Non-radiolabelled test substance 4-chloro-3-methylphenol (p-chloro-m-cresol, Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Water solubility: 3.4 g/L (at pH 7)
3.1.5	Method of analysis	HPLC method with UV detection: HPLC system: LaChrom / LaChrom Elite Column: RP18 (250*4 mm) Temperature: 25°C (oven) Detector: UV detector at 280 nm Injection volume: 25 or 50 µL Flow rate: 1.0 mL/min, Mobile phase: Acetonitrile / pure water, gradient mode
3.2	**Degradation products**	Degradation products tested: No
3.2.1	Method of analysis for degradation	Not relevant

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	products		
3.3	**Reference substance**	Not relevant	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Soil types**	Four soils were used which varied in clay content, organic carbon content and pH. Soil A: Loamy sand soil (pH 5.4, clay content 13.9 %, organic carbon content 2.29 %), origin: LUFA Speyer. Soil B: Sand soil (pH 5.1, clay content 8.5 %, organic carbon content 0.88 %), origin: LUFA Speyer. Soil C: Clay soil (pH 7.2 ± 0.1, clay content 65.1 ± 2.7 %, organic carbon content 1.75 ± 0.11 %), origin: LUFA Speyer. Soil D: Loam soil (pH 6.2, clay content 17.0 %, organic carbon content 3.0 %), origin: IRMM Geel	
3.5	**Testing procedure**		
3.5.1	Test system	The purpose of this study was to evaluate the stability of the test item in soils under conditions of an adsorption / desorption test. After performance of an adsorption step the amount of test item in the soil and test vessel extracts were determined and the mass balance was calculated. Refer to attached Figure A7.1.3_1 for detailed description (page 6 of this study summary).	
3.5.2	Test solution and Test conditions	Temperature: 20° ± 2°C Aqueous solvent: 0.01 M CaCl2, solution was used as the aqueous solvent phase. Evaluation of test results: Refer to attached Figure A7.1.3_2 for detailed description of evaluation (page 7 of this study summary).	
3.6	**Test performance**		
3.6.1	Preliminary test	Not performed	
3.6.2	Screening test: Adsorption	Refer to attached Figure A7.1.3_1 for detailed description (page 6 of this study summary).	X
3.6.3	Screening test: Desorption	Refer to attached Figure A7.1.3_1 for detailed description (page 6 of this study summary).	X
3.6.4	HPLC-method	See Point 3.1.5	
3.6.5	Other test	The purpose of this study was to evaluate the stability of the test item in soils under conditions of an adsorption / desorption test. After performance of an adsorption step the amount of test item in the soil and	

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test vessel extracts were determined and the mass balance was calculated.

4 RESULTS

4.1	**Preliminary test**	Not performed
4.2	**Screening test: Adsorption**	Analytical method: The concentration of the test item was determined using HPLC method. The linearity of the analytical method was proved in the concentration range from 0.5 to 10.0 mg test item/L. The regression coefficient was determined to be at least 0.999. A typical calibration curve is shown in attached Figure A7.1.3_4 (page 9 of this study summary). Test results: Four each of four soils 10 g of the soil in combination with 25 mL aqueous solution was used. The equilibrium time was 48 h. The test item was stable under the test conditions and did not adsorb on the surface of the test vessel. From analysis of the control soils no interfering compounds were detected. In case of the soils Lufa 2.1 (sand soil) and Lufa 2.2 (loamy sand soil) 97 % and 81 % of the nominal test item amount could be analytically recovered after adsorption. In case of Eurosoil 3 (loam soil) the recovery rate was 72 % and slightly below the required value. However in the main test (IBACON Report No. 32323195) the mass balance in case of this soil resulted in 98 %. In case of the Lufa soil 6S (clay soil) the recovery rate was 59 % and thus clearly below the required value. The low recovery rate of the test item from this forth soil is assumed not to be consequence of an instability of the test item but of the insufficient extraction method. Conclusion: In case of three different soil types the mass balance is better than 80 %. This indicates that the test item is stable under the test conditions. The results for the mass balances are summarized in Table A7_1_3-1 (page 10 of this study summary)
4.3	**Screening test: Desorption**	Not performed
4.4	**Calculations**	Evaluation of test results: Refer to attached Figure A7.1.3_2 for detailed description of evaluation (page 7 of this study summary).
4.5	**Degradation product(s)**	Not relevant (see Point 3.2)

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The purpose of this study was to evaluate the stability of the test item in soils under conditions of an adsorption / desorption test. After performance of an adsorption step the amount of test item in the soil and test vessel extracts were determined and the mass balance was calculated. For each of four soils 10 g of the soil in combination with 25 mL aqueous solution was used. The equilibrium time was 48 h.	
5.2	**Results and discussion**	The test item was stable under the test conditions and did not adsorb on the surface of the test vessel. From analysis of the control soils no interfering compounds were detected. In case of the soils Lufa 2.1 (sand soil) and Lufa 2.2 (loamy sand soil) 97 % and 81 % of the nominal test item amount could be analytically recovered after adsorption. In case of Eurosoil 3 (loam soil) the recovery rate was 72 % and slightly below the required value. However in the main test (IBACON Report No. 32323195) the mass balance in case of this soil resulted in 98 %. In case of the Lufa soil 6S (clay soil) the recovery rate was 59 % and thus clearly below the required value. The low recovery rate of the test item from this forth soil is assumed not to be consequence of an instability of the test item but of the insufficient extraction method. Conclusion: In case of three different soil types the mass balance is better than 80 %. This indicates that the test item is stable under the test conditions. The results for the mass balances are summarized in Table A7_1_3-1 (page 9 of this study summary)	
5.2.1	Adsorbed a.s. [%]	Not relevant (HPLC screening test)	
5.2.2	K_a	Log k' = 0.251	X
5.2.3	K_d	Not relevant (HPLC screening test)	
5.2.4	Ka_{oc}	log Koc = 2.2 (Koc = 158.5)	X
5.2.5	Ka/Kd	Not relevant (HPLC screening test)	
5.2.6	Degradation products (% of a.s.)	Not relevant (see Point 3.2)	
5.3	**Conclusion**	In case of three different soil types the mass balance is better than 80 %. This indicates that the test item is stable under the test conditions.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	November 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

Figure A7_1_3-1: Details of test design

Soil / Solution Ratio:	10 g of the soils in combination with 25 mL aqueous solution corresponding to a soil/solution ratio of 1:2.5 was used.
Test Item Solution:	112.3 mg of the test item was dissolved in methanol and it was filled up to 50 mL using a volumetric flask. For performance of the test the stock solution was diluted to about 100 mg/L in 0.01 M $CaCl_2$ solution. The concentration of the test item in 0.01 M $CaCl_2$ solution was below the water solubility of the test item.
Preparation of the Test Soils:	The air dried soil samples were equilibrated by shaking with 22.5 mL of 0.01 M $CaCl_2$ solution overnight. Afterwards 2.5 mL of the diluted test item solution (100 mg/L) was added in order to adjust the final volume to 25 mL. The volume of the added stock solution did not exceed 10 % of the final 25 mL volume.
Performance of the Test:	The soil suspension was shaken. After a period of 48 h the contact time was finished and the samples were collected. Therefore, the suspensions was centrifuged and filtered to obtain a clear solution. The aqueous solutions and the soil samples were analysed.
Control Sample:	Control samples with only the test item solution (without soil) were subjected to precisely the same steps as the test system, in order to check the stability of the test item in $CaCl_2$ solution and its possible adsorption on the surface.
Blank Sample:	Blank run per soil with the maximum amount of soil and total volume of $CaCl_2$ solution was subjected to the same test procedure. This solution served as a background control during the analysis to detect interfering compounds or contaminated soils.
Replicates:	Two replicates per each test soil and one replicate per control and blank sample were run.
Analysis of Aqueous Solutions:	The concentration of the test item in the aqueous phase was determined using HPLC method. If necessary the samples were diluted.
Analysis of Soil Samples:	The soils were analysed for the test item. To do this, the aqueous phase was recovered as much as possible. 20 mL of mixture of acetonitrile/water were added to each soil. It was shaken for a period of 60 min using a reciprocal shaker. Afterwards it was centrifuged and decanted and the extraction was repeated using 20 mL acetonitrile. It was shaken for a period of 60 min using a reciprocal shaker. It was centrifuged and decanted. In case of soil C and D the extraction was repeated using 10 mL acetonitrile. The extracts were filtered (0.45 µm PTFE filter) and analysed using HPLC method.
Documentation:	Exact data of the application, all observations and the measurements obtained are documented in the raw data.

Figure A7_1_3-2: Details of test design

Analytical Results:	The concentration of the test item in the test samples was determined using external standard method. The calibration curve will be obtained by correlation of peak area of at least 5 different standard solutions with their corresponding concentration. The correlation was performed using a linear regression function given by equation (1): $y = a * x + b$ (1) where y = peak area of the analyte peak x = concentration of the analyte a = slope b = y-axis intercept
Mass Balance:	The mass balance is defined as the percentage of the test item which can be analytically recovered after an adsorption test versus the nominal amount of test item at the beginning of the test.
Stability of the Test Item:	The amount of the test item in the soil and the $CaCl_2$-extracts was determined and the mass balance was calculated. If the mass balance will be less than 90 %, the test item will be considered to be unstable in the time scale of the adsorption / desorption test.

Figure A7_1_3-3: Deviations to the study plan

Concerning:	2.2 Test Parameters and Conditions
According to Study Plan:	The dry weight content of the soil will be determined.
Deviation to Study Plan:	The dry weight content of the loamy sand soil was not determined.
Reason for the Deviation:	By error
Presumed Effect on the Study:	None, the recovered amount of test item was calculated using the recovered volume of solvents.
Concerning:	2.3 Test Design
According to Study Plan:	Approximately 50 mg of the test item will be dissolved in methanol and it will be filled up to 50 mL using a volumetric flask.
Deviation to Study Plan:	112.3 mg of the test item was dissolved in methanol and it was filled up to 50 mL using a volumetric flask.
Reason for the Deviation:	A higher concentrated stock solution was prepared to lower the amount of solvent in the test.
Presumed Effect on the Study:	None, as the test item could be dissolved and the solvent volume in the final test solution was lower than 10 %.
Concerning:	2.4 Analytical Method
According to Study Plan:	Approximately 100 mg of the test item will be dissolved in methanol and will be filled up to 100 mL using a volumetric flask.
Deviation to Study Plan:	Approximately 50 mg of the test item were dissolved in acetonitrile and it was filled up to 50 mL using a volumetric flask.
Reason for the Deviation:	Standard operation procedure was applied.
Presumed Effect on the Study:	None, as a balance of appropriate accuracy was used.
Concerning:	2.4 Analytical Method
According to Study Plan:	Injection Volume 50 to 100 µL
Deviation to Study Plan:	Injection Volume 25 µL
Reason for the Deviation:	By error
Presumed Effect on the Study:	None, same volume was used for analysis of all samples and standard solutions.

Figure A7_1_3-4: Typical calibration curve (analytical method)

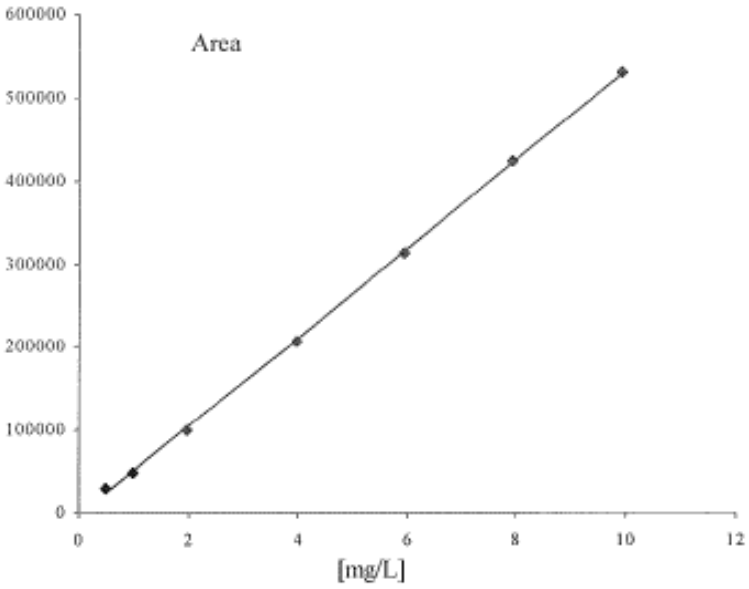


Table A7_1_3-1: Mass balance after extraction

Table 1. Mass Balance after Extraction

soil description	weight soil	water volume in soil	volume $CaCl_2$	volume test item	total volume	1st extraction				2nd extraction				3rd extraction				total mass	mass nominal in solution	mass balance
						removed volume	conc. found	mass found		removed solvent	conc. found	mass found		removed solvent	conc. found	mass found				
	[g]	[mL]	[mL]	[mL]	[mL]	[mL]	[µg/mL][1]	[µg][A]		[mL][2]	[µg/mL][1]	[µg][C]		[mL][2]	[µg/mL][1]	[µg][D]		[µg][1]	[µg][1]	[%][1]
Lufa 21	10.0	0.020	22.5	2.5	25	24	6.122	146.928		21	0.782	16.4		-	-	-		255.8	268	96%
Lufa 21	10.0	0.020	22.5	2.5	25	24	6.112	146.688		21	0.795	16.7		-	-	-		259.8	268	97%
Lufa 22	10.0	n.a.	22.5	2.5	25	22	2.758	60.676		23	1.275	29.3		-	-	-		212.2	268	79%
Lufa 22	10.0	n.a.	22.5	2.5	25	22	2.922	64.284		23	1.356	31.2		-	-	-		223.1	268	83%
Lufa 6S	10.0	0.200	22.5	2.5	25	21	2.555	53.655		20	1.101	22.0		15	0.490	7.4		158.1	268	59%
Lufa 6S	10.0	0.200	22.5	2.5	25	21	2.553	53.613		21	0.921	19.3		15	0.535	8.0		158.2	268	59%
Eurosoil 3	10.0	0.200	22.5	2.5	25	21	1.873	39.333		20	1.452	29.0		15	0.544	8.2		187.4	268	70%
Eurosoil 3	10.0	0.200	22.5	2.5	25	21	2.042	42.882		21	1.443	30.3		15	0.569	8.5		195.1	268	73%
Data from IBACON Project 32323195[3]						1st extraction ($CaCl_2$)				2nd extraction				3rd extraction				total	mass	
Eurosoil 3	10.1	0.117	22.5	2.5	25	20	2.870	57.4		40	3.583	143.3		-	-	-		235.2	250	94%
Eurosoil 3	10.2	0.119	22.5	2.5	25	19.5	2.717	52.9815		40	4.173	166.9		-	-	-		250.9	250	100%

[A] concentration found in removed volume $CaCl_2$ solution
[B] mass found after the 1st extraction with acetonitrile/water in removed volume solvent
[C] mass found after the 2nd extraction using acetonitrile in removed volume solvent
[D] mass found after the 3rd extraction using acetonitrile in removed volume solvent
[1] rounded value calculated from exact raw data
[2] after the last extraction step the remaining solvent in soil was added to the removed volume
[3] calculation of the mass balance according to the above used procedure
n.a. not available
- no 3rd extraction was performed
the removed volume after the adsorption step was estimated from numerous experiments

Section 7.2.3.2 **Annex Point IIIA 12.2.2**	**Fate and behaviour in soil** **Mobility**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	
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	27/10/2011	

Section 7.2.3.2 **Annex Point IIIA 12.2.2**	**Fate and behaviour in soil** **Mobility**
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 A7.3.1 Phototransformation in air (01)

Annex Point: IIIA 7.5 **(estimation method)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Anthe, M. (2006): p-Chloro-m-cresol. Calculation of indirect photodegradation. Dr. Knoell Consult GmbH, Leverkusen, Germany, Report No. KC-PD-04/06, Date: 2006-07-05.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Calculation based on QSAR	
2.2	**GLP**	Not applicable since the degradation behavior of p-chloro-m-cresol in air was calculated.	
2.3	**Deviations**	Not applicable since the degradation behavior of p-chloro-m-cresol in air was calculated.	
		3 MATERIALS AND METHODS	
		The half-life of p-chloro-m-cresol in air due to indirect photodegradation, i.e. oxidation of organic chemicals with photochemically produced hydroxyl radicals, was calculated using the software program AOPWIN (v. 1.91, 2000, US-EPA) based upon QSAR methods developed by Dr. Roger Atkinson and co-workers. AOPWIN requires the CAS No. or a chemical structure to make these predictions. Structures are entered into AOPWIN by SMILES (Simplified Molecular Input Line Entry System) notations. The estimation for p-chloro-m-cresol was carried out with respect to the OH radical reaction, using a 24-hours-day with 5×10^5 OH radicals/cm^3.	X
		4 RESULTS	
		The half-life of p-chloro-m-cresol in the troposphere is calculated to be 14.995 hours with a degradation rate of 25.4027×10^{-12} cm^3*$molecule^{-1}$*s^{-1}. This corresponds to a chemical lifetime in air of 0.625 days.	X
		5 APPLICANTS SUMMARY AND CONCLUSION	

Section A7.3.1 Phototransformation in air (01)

Annex Point: IIIA 7.5 **(estimation method)**

5.1	**Materials and methods**	The half-life of p-chloro-m-cresol in air due to indirect photodegradation, i.e. oxidation of organic chemicals with photochemically produced hydroxyl radicals, was calculated using the software program AOPWIN (v. 1.91, 2000, US-EPA) based upon QSAR methods developed by Dr. Roger Atkinson and co-workers.	
5.2	**Results and discussion**	The half-life of p-chloro-m-cresol in the troposphere is calculated to be 14.995 hours with a degradation rate of 25.4027 x 10^{-12} cm^3*$molecule^{-1}$*s^{-1}. This corresponds to a chemical lifetime in air of 0.625 days.	X
5.3	**Conclusion**	Due to the short chemical lifetime of p-chloro-m-cresol in air it is not to be expected that the compound will accumulate in air or be transported in the gaseous phase over long distances. Hence, air will not be an environmental compartment of concern.	
5.3.1	Reliability	[redacted]	X
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27 September 2011
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

Section 7.3.2 **Annex Point IIIA 12.3**	**Fate and behaviour in air** **Further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	
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	May 2009	
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 A7.3.2 Fate and behaviour in air (01)

Annex Point: IIIA 12.3

Official use only

1 REFERENCE

1.1	**Reference**	Gerharz, T. (2011): Neopredisan ® : Vaporisation behaviour of 4-chloro-3-methylphenol from an inert surface (glass petri dish). Lanxess Deutschland GmbH, Leverkusen, Germany. Report No. D 2010-22.1.5, date: 2010-04-19 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; Internal test procedure developed for this experiment and analytical method no. M-00769-02 [HPLC] "Determination of known phenolic compounds in disinfectants".
2.2	**GLP**	No
2.3	**Deviations**	---

3 MATERIALS AND METHODS

3.1	**Test material**	Neopresidan ® 135-1
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Neopresidan ® 135-1 has a nominal content of 4-chloro-3-methylphenol of about [redacted]. [redacted]
3.2	**Testing procedure**	
3.2.1	Test system	This experiment should show how the active ingredient 4-chloro-3-methylphenol (CMK) contained in Neopredisan® 135-1 evaporates from a surface. For this experiment a glass surface was chosen as an example of an inert surface. The test period was 96 hours. Neopredisan® 135-1 was dissolved at a rate of 2% in demineralised water and an amount of 6.2 mL of the mixture was put on several glass petri dishes for parallel drying in the air. For the vaporisation the petri dishes were stored in the laboratory on a free desk surface (working area). At the free desk surface an air exchange rate of 8 (= 25 m^3/m^2 x h) should be considered as this is required for laboratories according to the German Standard.

Section A7.3.2 Fate and behaviour in air (01)

Annex Point: IIIA 12.3

3.2.2	Test concentration	According to DVG (German Veterinarian Society) a disinfectant quantity of 400 mL/m^2 may be applied for a proper disinfection. A typical disinfection solution was according to DVG guidelines applied with 400 mL/m^2. Therefore we need 6.16 mL on the surface of the petri dish which has 154 cm^2 = 0.0154 m^2. The 4-chloro-3-methylphenol amount in the 100 mL measuring piston (for detail see description of the test procedure) corresponds to the surface of the petri dish (154 cm^2).	X
3.2.3	Duration of the test	96 hours	
3.3	**Examination**	At each test time temperature and humidity were determined. At each test time a petri dish was rinsed with 2 x 30 mL Isopropanol. The solutions were collected in a 100 mL volumetric flask. To make sure that no product was lost the used funnel as well as the neck of the volumetric flask was rinsed with 10 mL Isopropanol each and the volumetric flask was filled up with Isopropanol. The 4-chloro-3-methylphenol amount of this sample was analysed with a validated analytical method.	
		4 RESULTS	
		After 96 hours the 4-chloro-3-methylphenol quantity left on the surface was <1% of the amount originally applied.	X
		5 APPLICANTS SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	An aqueous solution containing 4-chloro-3-methylphenol was investigated for the vaporisation behaviour from an inert glass surface (petri dish).	X
5.2	**Results and discussion**	After 96 hours the 4-chloro-3-methylphenol quantity left on the surface was <1% of the amount originally applied.	X
5.3	**Conclusion**	It is clearly shown that the active ingredient 4-chloro-3-methylphenol evaporates consistently from the glass surface.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27/09/2011
Materials and Methods	[redacted]
Results and discussion	[redacted]

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

	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	

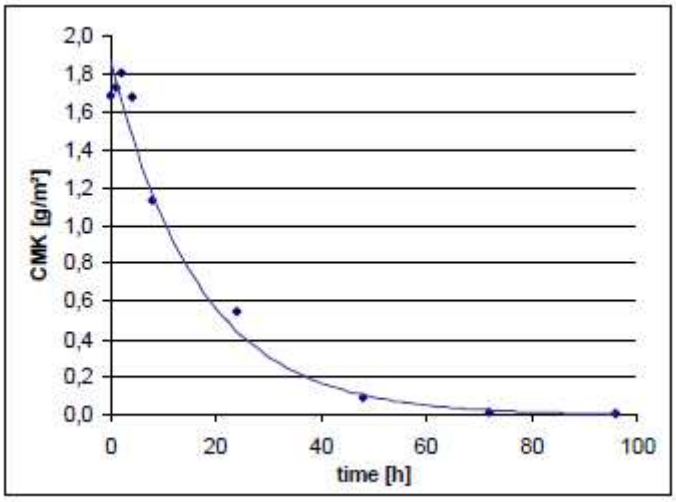
Table A7_3_2-1: Test conditions

Time [hours]	Temperature [°C]	Humidity [%]
0	18.7	32.7
1	18.7	32.6
2	18.7	32.7
4	18.7	32.8
8	18.7	32.7
24	19.1	32.9
48	19.1	32.7
96	19.1	32.7

Table A7_3_2-2: Evaporation results

Time [hours]	[g CMK/m^2]	Residual % CMK
0	1.69	100
1	1.73	102.39
2	1.81	107.18
4	1.68	99.31
8	1.14	67.28
24	0.54	32.20
48	0.09	5.54
72	0.01	0.78
96	0.01	0.54

Figure A7_3_2-1: Evaporation of 4-chloro-3-methylphenol from the surface of a glass petri dish



Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] Acute Toxicity of Preventol CMK Technical to the Rainbow Trout (*Oncorhynchus mykiss*) Under Static Renewal Conditions. [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes U.S.-EPA FIFRA § 72-1
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Preventol CMK Technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Gas chromatograph (GC) equipped with a flame ionisation detector (FID) A Report about analytical methods and analytical results (ABC Laboratories, Report No. 404491, Date: 1993-02-12) is attached to the original report.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_1-1
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference	-

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

	substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	Rainbow trout (*Oncorhynchus mykiss*), see table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4 The exposure of rainbow trout to Preventol CKM was conducted under static-renewal conditions. Five concentrations of the test material, dilution water control and solvent control were used for the test. An initial range finding test was conducted to determine the concentration levels to use in the definitive test. A first definitive study was cancelled after 72 hours due to lack of mortality. Based upon the range find data and the results from the first test, the concentrations for a second study were determined.
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality; Sublethal and behavioural responses (observations)
3.4.7	Sampling	Survival of fish was monitored daily and dead fish were removed. Observations for sublethal and behavioural effects were also made. Fish from control and solvent control chambers were weighed and measured at test termination. Temperature, conductivity, dissolved oxygen and pH values were measured at 0, 48 and 96 hours of testing in each aquarium. Measurements at 48 hours were taken in the old and the fresh solutions. Temperature was measured daily.
3.4.8	Monitoring of TS concentration	Yes, analytical measurements were performed at day 0 and at day 2. Analyses at day 2 were done for the old and the fresh test solutions. In case 100% mortality was reached in test concentrations prior to the end of the test, the analytical determinations were made at that time.
3.4.9	Statistics	The 24, 48, 72 and 96 – hour LC_{50} values and their corresponding 95% confidence limits were calculated by a computerized program developed by Stephan, C.E. (1977) using one of three statistical techniques: moving average angle, binomial probability and probit analysis. The appropriate method was determined on the basis of data characteristics as described in: Stephan, C.E. (1977): Methods for Calculating an LC_{50}. In: Mayer, FL. & Hamelink, J.L. (Eds.): Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA, pp. 65-84.
		4 RESULTS

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

4.1	**Limit Test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		X
4.2.1	Initial concentrations of test substance	Nominal concentrations: control, solvent control (acetone) and 0.259, 0.432, 0.72, 1.2 and 2.0 mg a.i./L	
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): control, solvent control and 0.218, 0.366, 0.644, 1.110 and 2.039 mg a.i./L. The mean measured concentrations of Preventol CMK during the test period ranged from 84 - 102 % of nominal. No undissolved test substance was observed in the test chambers. All reported results are based on mean measured concentrations of the test substance.	X
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7	
4.2.4	Concentration / response curve	A concentration/ response curve is not given in the report.	
4.2.5	Other effects	See table A7_4_1_1-6 for detailed description of observed responses.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	There were neither mortalities nor symptoms of intoxication in the dilution water or solvent control group.	
4.3.2	Nature of adverse effects	-	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A 96 - hour static-renewal study was conducted in accordance with U.S.-EPA FIFRA Guideline § 72-1 in order to estimate the acute toxicity of Preventol CMK to rainbow trout (*Oncorhynchus mykiss*).	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Oncorhynchus mykiss*

5.2	**Results and discussion**	A 96 - hour LC_{50} value was calculated to be 0.917 mg a.i./L with 95% confidence limits ranging from 0.644 to 1.110 mg/L. Behavioural or sublethal effects were observed during the exposure period. All fish at 0.644 mg/L were observed to be hyperreactive. Survivors in the 1.11 mg/L level showed a loss of equilibrium and vertical orientation. The No observed effect concentration (NOEC) was 0.366 mg/L based upon the lack of mortality and sublethal effects at this concentration. All results are based on the mean measured test concentrations of the test substance.
5.2.1	96h-LC_0	0.366 mg a.i./L (NOEC)
5.2.2	96h-LC_{50}	0.917 mg a.i./L
5.2.3	96h-LC_{100}	2.039 mg a.i./L
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_1-8. The test is considered as valid and the results are used in the environmental risk assessment.
5.3.1	Other Conclusions	-
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	-
Vehicle	Acetone
Concentration of vehicle	50 µL acetone/L water for all test levels and solvent control
Vehicle control performed	Yes
Other procedures	-

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Process water (spring water blended with treated city water)
Alkalinity	47 mg/L (as $CaCO_3$)
Hardness	52 mg/L (as $CaCO_3$)
pH	7.7
Oxygen content	-
Conductance	132 µmhos
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	The 20 days old eggs were delivered on 1992-11-11. Mean body wet weight at the beginning of the test was 0.29 (± 0.08) g and mean body standard length was 2.91 (± 0.22) cm. The biomass loading was 0.34 g fish/L test medium.
Kind of food	During the acclimation period fish were fed daily with newly hatched brine shrimp and/or a commercial fish food.
Amount of food	Not reported
Feeding frequency	Daily
Pretreatment	During 48 hours immediately prior to test initiation fish were held under test conditions (temperature 12.1 C, 16 h photoperiod, no food).
Feeding of animals during test	Fish were not fed 48 h before and during the study.

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static-renewal
Renewal of test solution	Renewal of the test solution on day 2
Volume of test vessels	20 L stainless steel aquaria filled to a 17 L volume
Volume/animal	0.85 L
Number of animals/vessel	20
Number of vessels/ concentration	One vessel
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	11.8 – 12.7 °C (minimum - maximum during the exposure period)
Dissolved oxygen	7.1 - 10.2 mg/L (71 - 95 % saturation)
pH	7.1 – 7.6
Adjustment of pH	Not stated
Aeration of dilution water	Test solutions were not aerated during the study.
Intensity of irradiation	50 -70 foot-candles, combination of cool white and Agro-Lite fluorescent bulbs.
Photoperiod	16 hours light and 8 hours dark, (with 30-minute transition period, simulating dawn and dusk)

Table A7_4_1_1-6: Cumulative mortality and behavioural observations

Dose[1] (mg a.i./L)	Exposure time							
	24 h		48 h		72 h		96 h	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	20 N	0	20 N	0	20 N	0	20 N
Solvent control	0	20 N	0	20 N	0	20 N	0	20 N
0.218	0	20 N	0	20 N	0	20 N	0	20 N
0.366	0	20 N	0	20 N	0	20 N	0	20 N
0.644	0	20 H	0	20 H	0	20 H	0	20 H
1.110	17	1 N 2 LE	17	3 E, LE	17	1 OB 1 VO 1 E, LE	17	2 LE,VO 1 VO
2.039	20	0 N	20	0 N	20	0 N	20	0 N

[1] Effect data are based on mean measured concentrations

Abbreviations of behavioural observations

N Normal

H Hyperreactive

E Erratic behaviour

VO Vertical orientation

LE Loss of equilibrium

OB On bottom

Table A7_4_1_1-7: Calculated LC_{50} values (based on mean measured concentrations)

Exposure period (h)	LC_{50} (mg test substance/l) [1]	95 % C.I. (mg test substance/l)	Method of statistical calculation
24 h	0.917	0.644 - 1.110	Binominal Probability Method
48 h	0.917	0.644 - 1.110	Binominal Probability Method
72 h	0.917	0.644 - 1.110	Binominal Probability Method
96 h	0.917	0.644 - 1.110	Binominal Probability Method

[1] Effect data are based on measured concentrations

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fulfilled
Mortality of control animals <10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥ 80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances	Not applicable	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

Official use only

		1 REFERENCE
1.1	**Reference**	Gagliano, G.G. & Bowers, L.M. (1993): Acute Toxicity of Preventol CMK technical to the Waterflea (*Daphnia magna*) under static conditions. Miles Incorporated, Agriculture Division, South Metcalf, Stilwell, Kansas, US, unpublished report No. 105021, Date: 1993-02-19.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, U.S.-EPA FIFRA § 72-2: "Acute toxicity test for freshwater invertebrates"
2.2	**GLP**	Yes
2.3	**Deviations**	None
		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
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Gas chromatograph (GC) equipped with a flame ionisation detector (FID) Report about analytical methods and analytical results (ABC Laboratories, Report No. 404492, Date: 1993-02-12) is attached to the original report.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1
3.3	**Reference substance**	-
3.3.1	Method of analysis for reference	-

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

	substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	X
3.4.2	Test organisms	See table A7_4_1_2-3	
3.4.3	Test system	See table A7_4_1_2-4	
3.4.4	Test conditions	See table A7_4_1_2-5	
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Mortality and behavioural observation	
3.4.7	Sampling	Mortality and behavioural observations were performed daily and dead Daphnia were removed. Conductivity, pH, alkalinity, hardness and dissolved oxygen were measured in the control, solvent control, low, middle and high concentrations containing surviving daphnids at 0 and 48 hours of testing. Temperature was measured daily.	
3.4.8	Monitoring of TS concentration	Yes, analytical measurements of test substance at 0 and 48 hours; see table A7_4_1_2-6	
3.4.9	Statistics	Statistical analysis was obtained by employing a computerized program. The LC_{50} and EC_{50} values were calculated using one of the following statistical methods: binomial probability, moving average angel and probit, depending on the characteristics of the data..	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 1.0, 2.0, 3.0, 4.0 and 5.0 mg a.i./L (an untreated and solvent control were run in parallel)	X
4.2.2	Actual concentrations of test substance	Mean measured concentrations: 0.88, 1.73, 2.67, 3.90 and 5.52 mg a.i./L The mean measured concentrations during the rest period ranged from 87 to 110 % of nominal (see table A7_4_1_2-6. No undissolved Preventol CMK was observed in the test chambers. These results indicate that the test concentrations prepared for this test correspond to nominal concentrations and that the test substance was stable for the duration of the study. All results based on mean measured	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

		concentrations.	
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-7 and table A7_4_1_2-8. No mortality occurred in the 0.88, 1.73 and 2.67 mg/L groups, 50% in the 3.90 mg/L concentration and 100% in the highest tested concentration of 5.52 mg/L. Sublethal effects were observed in the measured concentrations of 2.67, 3.90 and 5.52 mg/L. Based on mortality/sublethal effects the 48 h EC_{50} was 2.29 mg/L with 95% confidence limits 1.73 to 2.67 mg a.i./L. The 48 h LC_{50} was 3.90 mg/L based on mortality data. The NOEC was determined to be 1.73 mg/L based upon the lack of mortality and sublethal effects at this concentration.	
4.2.4	Concentration / response curve	Not provided in the report	
4.2.5	Other effects	No other effects observed apart from those mentioned above.	
4.3	**Results of controls**	No mortality or abnormal behaviour occurred in the control groups.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Acute toxicity test to *Daphnia magna* was performed in accordance with US-EPA, Pesticide Assessment Guidelines, Series 72-2 (Acute Toxicity Test for Freshwater Aquatic Invertebrates). The test animals were exposed under static conditions to measured Preventol CMK concentrations of 0.88, 1.73, 2.67, 3.90 and 5.52 mg a.i./L, an untreated and solvent control were run in parallel. After 24 and 48 hours, the inability to swim and/or the immobility of the animals was determined.	
5.2	**Results and discussion**	No mortality occurred in the 0.88, 1.73 and 2.67 mg/L groups, 50% in the 3.90 mg/L concentration and 100% in the highest tested concentration of 5.52 mg/L. Sublethal effects were observed in the measured concentrations of 2.67, 3.90 and 5.52 mg/L. Based on mortality/sublethal effects the 48 h EC_{50} was 2.29 mg/L with 95% confidence limits 1.73 to 2.67 mg a.i./L (based on mean measured concentrations). No mortality or behavioural/sublethal effects occurred in the control groups.	
5.2.1	NOEC	1.73 mg a.i./L	
5.2.2	EC_{50}	2.29mg a.i./L	X
5.2.3	EC_{100}	3.9 mg a.i./L (100% effected)	
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_2-9. All validity criteria are fulfilled by the study and the results can be considered as valid.	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	A slight deficiency in reporting was obvious as the solvent substance is not specified in the report. However, this is considered not to influence the reliability of the study.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	-
Vehicle	Not reported
Concentration of vehicle	Not reported
Vehicle control performed	Yes
Other procedures	-

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Process water (spring water blended with treated city water)
Alkalinity ($CaCO_3$)	~~135~~ **134** mg/L
Hardness ($CaCO_3$)	~~167~~ **164** mg/L
pH	8.0 - 8.2
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	8.6 – 9.1 mg/L
Conductance	344 µmhos
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*, Lot No. ABS092490
Source	The strain was obtained from Aquatic BioSystems, Fort Collins, Colorado.
Age (at start of the study)	≤ 24 – hours old neonates
Breeding method	Daphnia culture techniques were based on those described by USEPA (1985), A subculture of brood carrying daphnids was isolated on Day -1 for the Day 0 collection of ≤ 24 h old neonates
Kind of food	The animals were fed daily with algae (*Scenedesmus subspicatus* and *Ankistrodemus falcatus*) and /or a yeast/troutchow/cerophyll™ mixture.
Amount of food	Not specified
Feeding frequency	Daily
Pre-treatment	-
Feeding of animals during test	Daphnia were not fed during the test.

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	The test was performed under static conditions
Volume of test vessels	2 L glass beakers; each test vessel was filled to an approx. volume of 1 L
Volume/animal	100 mL
Number of animals/vessel	10
Number of vessels/ concentration	2 replicates per concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	19.8 - 19.9 °C
Dissolved oxygen	8.6 – 9.1 mg/L (95 - 100% saturation at 20°C)
pH	8.0 - 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	The light intensity was about 60 - 65 footcandles (corresponding to approx. 645 - 700 lux)
Photoperiod	16:8 light-dark cycle (16-h daylight photoperiod)

Table A7_4_1_2-6: Measured test substance concentrations in test solutions

Nominal conc. [mg a.i./L]	Measured concentrations [mg a.i./L]			% of nominal
	0 Hour	48 Hour	Mean (± SD)	
Control	< 0.1	< 0.1	-	-
Solvent control	< 0.1	< 0.1	-	-
1.0	1.03	0.72	0.88 (0.17)	88
2.0	1.89	1.56	1.73 (0.18)	87
3.0	2.72	2.61	2.67 (0.06)	89
4.0	4.20	3.59	3.90 (0.34)	98
5.0	5.52	*	5.52	110

SD = Standard deviation
* No surviviors, therefore no sample set

Table A7_4_1_2-7: Cumulative mortality and behavioural observations (after 24 and 48 h)

Mean measured Concentration [mg a.i./L]	Effects on *Daphnia magna*			
	24 hours		48 hours	
	Dead	Obs.	Dead	Obs.
Control	0	20 N	0	20 N
Solvent control	0	20 N	0	20 N
0.88	0	20 N	0	20 N
1.73	0	20 N	0	20 N
2.67	0	16 N 4 OB	0	3 N 16 OB 1 OB, VLM
3.90	0	8 N 12 OB	10	10 OB, VLM
5.52	6	14 OB	20	0

Abbreviations of behavioural observations

N Normal

OB On bottom of vessel

VLM Very little movement

Table A7_4_1_2-8: Calculated EC_{50} values for *Daphnia magna* exposed to Preventol CMK

Exposure period [h]	EC_{50} [mg a.i./L]	95 % C.I. [mg a.i./L]	Method of statistical calculation
24 h	3.45	3.09 - 3.83	Probit
48 h	2.29	1.73 - 2.67	Binominal Probability

Table A7_4_1_2-9: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled	Not fulfilled
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**X**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**X**	
Concentration of test substance ≥ 80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances	**Not applicable**	

Section A7.4.1.3 Growth inhibition test on algae(01_02)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

Official use only

		1 REFERENCE
1.1	**Reference**	Caspers, N. (1983/1991): Preventol CMK (4-chloro-3-methyl-phenol) – Growth Inhibition Test Algae. Bayer AG, WV-Umweltschutz, Leverkusen, Germany, Report (unpublished), Date: 1991-01-28 (test was performed in May 1983). Weyers, A. (2006): Preventol CMK – Algae, Growth Inhibition Test. Re-Evaluation based on Study Report Growth Inhibition Test Algae (1983) and the corresponding raw data. Bayer Industry Services, Leverkusen, Germany, Report (unpublished), Date: 2006-07-07.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, ISO Draft Method (147/SC 5/WG 5 N 67) “Determination of toxicity with Algae” (1982); this ISO Draft Guideline is comparable to OECD Guideline 201 (adopted 1984)
2.2	**GLP**	GLP was not compulsory at time of testing
2.3	**Deviations**	The maintenance of test substance concentrations was not proved by analytical measurements.
		3 MATERIALS AND METHODS
3.1	**Test material**	4-chloro-3-methylphenol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Test substance concentrations are not confirmed by analytical method.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference**	No

Section A7.4.1.3 Growth inhibition test on algae(01_02)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

	substance		
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Culture medium	The nutrient medium was prepared according to Bringmann and Kühn (1977). The test suspensions contain the following (based on 1 litre): 15 mg NH_4Cl, 12 mg $MgCl_2$ x$6H_2O$, 18 mg $CaCl_2$ x$2H_2O$, 15 mg $MgSO_4$ x$7H_2O$, 1.6 mg KH_2PO_4, 80 µg $FeCl_3$ x$6H_2O$, 100 µg Na_2EDTA x$2H_2O$, 185 µg H_3BO_3, 415 µg $MnCl_2$ x$4H_2O$, 3 µg $ZnCl_2$, 1.5 µg $CoCl_2$ x$6H_2O$, 0.01 µg $CuCl_2$ x$2H_2O$, 7 µg Na_2MoO_4 x$2H_2O$. Solid $NaHCO_3$, is added to make up a final concentration of 50 mg/l. The composition of the nutrient medium is described in detail in the Re-evaluation report (Weyers, 2006; pages 9 and 22).	
3.4.2	Test organisms	See table A7_4_1_3-1	X
3.4.3	Test system	See table A7_4_1_3-2	
3.4.4	Test conditions	See table A7_4_1_3-3	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Effects of p-Chloro-m-cresol on the growth of the green alga *Desmodesmus subspicatus*: - The test substance concentration at which there was 50 % inhibition of growth of biomass (E_bC_{50}) and - The test substance concentration at which there was 50% inhibition of the growth rate (E_rC_{50}) Also detected were the lowest concentration at which there was an observable effect (LOEC) and the concentration at which there was no observed effect (NOEC).	
3.4.7	Sampling	Samples to determine the number of algae/ml suspension were taken at 24 and 48 hours.	X
3.4.8	Monitoring of TS concentration	No. The test substance concentrations was not proved by analytical measurements.	
3.4.9	Statistics	The EC_{50} values for growth of biomass (E_bC_{50}) and for algal growth rate (E_rC_{50}) were calculated using probit analyses after: Finney, D.J. (1952): Statistical Methods in Biological Assay, London. The NOEC and LOEC values were calculated by a multisampling comparison according to Dunnett 1955[1)] and 1964[2)]. 1): Dunnett, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. Amer. Statist. Ass. J., 50, pp. 1096-1121. 2) Dunnett, C.W. (1955): New tables for multiple comparisons with a control.	

Section A7.4.1.3 Growth inhibition test on algae(01_02)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

		Biometrics, 20, pp. 482-491.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations: 1.0, 3.2, 10.0 and 32.0 mg/L
4.2.2	Actual concentrations of test substance	Test results are based on nominal test substance concentrations.
4.2.3	Growth curves	Growth curves (number of cells vs. time) are given in the re-evaluation report (p. 21).
4.2.4	Concentration / response curve	Growth inhibition curves (effect of test substance on amount of algal biomass vs. test substance concentration as well as effect of test substance on the algal growth rate vs. test substance concentration, respectively) are plotted in the report (pp. 19-20).
4.2.5	Cell concentration data	See table A7_4_1_3-4
4.2.6	Effect data (cell multiplication inhibition)	See table A7_4_1_3-5, Table A7_4_1_3-6 and Table A7_4_1_3-7
4.2.7	Other observed effects	--
4.3	**Results of controls**	See table A7_4_1_3-5 and Table A7_4_1_3-6
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	--
4.4.2	Results	--
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The influence of p-Chloro-m-cresol on the growth of the green alga *Desmodesmus subspicatus* was investigated in a 72 h hours static test according to ISO Draft Method (147/SC 5/WG 5 N 67) "Determination of toxicity with Algae" (1982); this ISO Draft Guideline is comparable to OECD Guideline 201 (adopted 1984)

Section A7.4.1.3 Growth inhibition test on algae(01_02)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

5.2	**Results and discussion**		
5.2.1	NOEC	NOE_bC = 3.20 mg/L;	
		NOE_rC = 3.20 mg/L	
5.2.2	EC_{50}	E_bC_{50} = 5.09 mg/L;	
		E_rC_{50} = 11.12 mg/L	
5.3	**Conclusion**	Validity criteria are summarised in table A7_4_1_3-8.	X
		Dose – response relationship: a clear dose – response relationship can be derived from the cell concentration data.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	Nominal test substance concentrations were not confirmed by analytical measurements during the test.	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

Remarks	[redacted]
	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 A7_4_1_3-1: Test organisms

Criteria	**Details**
Species	Planktonic freshwater algal species *Desmodesmus subspicatus* (former name: *Scenedesmus subspicatus)*
Strain	Non-axenic strain
Source	Obtained from "The Collection of Algal Cultures" of the Institute for Plant Physiology, University Goettingen, Germany
Laboratory culture	Yes
Method of cultivation	**Stock cultures** of the alga were grown at 23 ± 2 °C at a light intensity in the range 60-120 µE x m^{-2} s^{-1} in 300 ml Erlenmeyer flasks containing 50 ml nutrient solution 1 (see Point 3.4.1; Culture medium) **Pre-cultures** of the alga were inoculated with 10,000 cells/ml. These were grown in ~~200 ml~~ nutrient solution (see Point 3.4.1; Culture medium) for three days in an incubator and then used to prepare treated and control cultures for growth inhibition tests. **Test cultures and the cell-free culture media** used for quantitative analyses were prepared by mixing the appropriate quantities of the following components in the following order: - sterile, deionized water - 10-fold concentrated nutrient solution - stock solution of test substance. The cultures were used for the growth inhibition tests by inoculating it with enough 3-day old pre-culture to give a density of 10,000 cells/ml. All operations were done under sterile conditions.
Pre-treatment	-
Initial cell concentration	Test started with a biomass of 10,000 (= 1 x 10^4) cells per ml nutrient solution

Table A7_4_1_3-2: Test system

Criteria	Details
Volume of culture flasks	300 ml Erlenmeyer flasks
Culturing apparatus	Incubation was performed under standardised conditions according to the mentioned guidelines. Light chamber in which a temperature in the range 21 to 25°C was maintained at ± 2°C; occasionally the test cultures were shaken by hand.
Light quality	Continuous illumination at 120 µE x m^{-2} s^{-1} ± 20 µE;
Procedure for suspending algae	No information available.
Number of vessels/ concentration	Control: 3 flasks; Each of 4 test substance concentrations: 3 flasks
Test performed in closed vessels due to significant volatility of TS	Yes

Table A7_4_1_3-3: Test conditions

Criteria	Details
Test temperature	23 ± 2 °C
pH	pH values not reported
Aeration of dilution water	No data
Light intensity	120 µE x m^{-2} s^{-1} ± 20 µE
Photoperiod	Continuous illumination in the incubator (24 h/day)

Table A7_4_1_3-4: Cell numbers at different test substance concentrations during test (average* values)

Nominal concentration (mg/L)	Average Cell Number (initial cell density: 10,000 cells/mL)		
	After 24 h	After 48 h	After 72 h
Control	45,556	158,889	603,333
1.0	40,000	183,333	737,778
3.2	42,222	123,333	442,222
10.0	11,111	35,556	111,111
32.0	8,889	10,000	8,889

* Number of samples: control: 3 vessels; each test substance concentration level: 3 vessels

Table A7_4_1_3-5: Results for mean growth (integral of biomass): Areas under the growth curves, inhibition/increase of cell growth (in %) of *Desmodesmus subspicatus* at different test substance concentrations, and results of Multiple t-Tests according to Dunnett (1955)

	Test results		Results of Dunnett Multiple t-Test*					
Nominal concentration (mg/L)	Area under growth curve A	Inhibition (+) / Increase (-) %	$S_{biomass}$	t	$t_{0.05}$	Signif.	$t_{0.01}$	Signif.
Control	481,111	0.0	18,733	--	--	--	--	--
1.0	566,667	-17.9	213,099	1.41	-2.39	-	-3.27	-
3.2	361,667	24.8	32,787	-1.98	-2.39	-	-3.27	-
10.0	77,222	83.9	11,706	-6.68	-2.39	+	-3.27	+
32.0	-1,667	100.3	6,009	-7.98	-2.39	+	-3.27	+

* Significance levels = 5 % and 1 %

Table A7_4_1_3-6: Results for mean growth rate: Specific growth ratesAreas under the growth curves, inhibition/increase of cell growth (in %) of *Desmodesmus subspicatus* at different test substance concentrations, and results of Multiple t-Tests according to Dunnett (1955)

	Test results		Results of Dunnett Multiple t-Test*					
Nominal concen-tration (mg/L)	**Growth rate**	**Inhibition (+) / Increase (-)**						
	(1/d)	**%**	**$S_{growth\ rate}$**	**t**	**$t_{0.05}$**	**Signif.**	**$T_{0.01}$**	**Signif.**
Control	1.37	0.0	0.031	--	--	--	--	--
1.0	1.41	-3.3	0.159	0.79	-2.39	-	-3.27	-
3.2	1.26	7.7	0.049	-1.23	-2.39	-	-3.27	-
10.0	0.80	41.5	0.055	-6.68	-2.39	+	-3.27	+
32.0	-0.09	106.6	0.244	-16.64	-2.39	+	-3.27	+

* Significance levels = 5 % and 1 %

Table A7_4_1_3-7: Summary of the results from a 72 h growth inhibition test with p-Chloro-m-cresol and *Desmodesmus subspicatus*

Inhibition-Parameter	Endpoint	Value (mg a.s./L)
Biomass (72 h)	E_bC_{50}	5.09
	LOE_bC	10.0 ($t_{\alpha\ 0.05}$)
	NOE_bC	3.20 ($t_{\alpha\ 0.05}$)
Growth Rate (72 h)	E_rC_{50}	11.12
	LOE_rC	10.0 ($t_{\alpha\ 0.05}$)
	NOE_rC	3.20 ($t_{\alpha\ 0.05}$)

Table A7_4_1_3-8: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥ 80% of initial concentration during test	Results based on nominal values	

Criteria for poorly soluble test substances	Not applicable	-

Section A7.4.1.3 Growth inhibition test on algae(03)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

Official use only

1 REFERENCE

1.1	**Reference**	Vinken, R. and Wydra, V. (2007): Toxicity of 4-Chloro-3-methylphenol (Preventol CMK) to *Desmodesmus subspicatus* in an Algal Growth Inhibition Test. Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany, Project No. 32324210 date: 2007-01-04 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Commission Directive 92/69/EEC, Annex Part C, C.3: "Algal Inhibition Test", Official Journal of the European Communities No. L 383 A, dated December 29, 1992. OECD Guideline for Testing of Chemicals, Section 2, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test", adopted March 23, 2006.
2.2	**GLP**	GLP
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	4-Chloro-3-methylphenol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	The quantification of the test item was performed using liquid chromatography (HPLC-method)
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference	-

Section A7.4.1.3 Growth inhibition test on algae(03)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

	substance		
3.4	**Testing procedure**		
3.4.1	Culture medium	Reconstituted Water (OECD Medium): Analytical grade salts were added at the following nominal concentrations in deionised water (conductivity <5 µScm^{-1}). The composition of the nutrient medium is described in detail in the report (page 15).	X
3.4.2	Test organisms	See table A7_4_1_3-1	
3.4.3	Test system	See table A7_4_1_3-2	
3.4.4	Test conditions	See table A7_4_1_3-3	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Effects of p-chloro-m-cresol on the growth of the green alga *Desmodesmus subspicatus*: - The test substance concentration at which there was 50% inhibition of yield (E_yC_{50}) - The test substance concentration at which there was 50 % inhibition of growth of biomass (E_bC_{50}) and - The test substance concentration at which there was 50% inhibition of the growth rate (E_rC_{50}) Also detected were the lowest concentration at which there was an observable effect (LOEC) and the concentration at which there was no observed effect (NOEC).	
3.4.7	Sampling	Defined volumes of the algae suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The cell densities in the samples were determined by spectrophotometrical measurement. The pH-values of the test media were measured in samples from all test item concentrations and the control at the start and at the end of the test. The temperature was measured daily in an Erlenmeyer flask filled with water and incubated under the same conditions as the test flasks. The light intensity was measured once during the test.	X
3.4.8	Monitoring of TS concentration	The concentrations of the test item 4-chloro-3-methylphenol (Preventol CMK) were analysed in the duplicate test media samples from all test concentrations from both sampling times (0 and 72 hours). From the control samples only one of the duplicate samples was analysed from each of both sampling times.	X
3.4.9	Statistics	Based on the calculated cell densities, the E_rC_{50}, E_bC_{50}, and E_yC_{50} and the corresponding EC_{10} values and their 95%-confidence limits were calculated by Probit analysis. For the determination of the LOEC and NOEC, the calculated growth rates, areas under the growth curve and yields at the test concentrations were tested on significant differences to the control values by the Bonferroni t-test (growth rate, area under the growth curve) and Williams test (yield), respectively (ToxRat Version 2.09, 2001-2005).	
		4 RESULTS	
4.1	**Limit Test**	Not performed	

Section A7.4.1.3 Growth inhibition test on algae(03)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

4.1.1	Concentration	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	The concentrations were selected according to the results of a range finding test. Nominal concentrations: 0.95, 3.1, 9.8, 31, and 100 mg test item/L	
4.2.2	Actual concentrations of test substance	At the start of the test 97 % of the nominal test concentrations were found (average for all test concentrations). After 72 hours test duration 89 % of the nominal values were determined (average for all test concentrations). Thus, during the test period of 72 hours the algae were exposed to a mean of 93 % of nominal. Therefore, all reported results arc related to nominal concentrations of the test item.	
4.2.3	Growth curves	Growth curves (number of cells vs. time) are given in report (p. 34)	
4.2.4	Concentration / response curve	Growth inhibition curves (effect of test substance on amount of algal biomass vs. test substance concentration as well as effect of test substance on the algal growth rate vs. test substance concentration, respectively) are not given in the report.	
4.2.5	Cell concentration data	See table A7_4_1_3-4	
4.2.6	Effect data (cell multiplication inhibition)	See table A7_4_1_3-5, Table A7_4_1_3-6, Table A7_4_1_3-7 and Table A7_4_1_3-8	
4.2.7	Other observed effects	--	X
4.3	**Results of controls**	See table A7_4_1_3-5 to Table A7_4_1_3-8	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	--	
4.4.2	Results	--	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The purpose of this test was to determine the inhibitory effect of the test item 4-chloro-3-methylphenol (Preventol CMK) on the growth of the freshwater green algal species *Desmodesmus subspicatus*. Exponentially growing cultures of this unicellular green algal species were exposed to various concentrations of the test item (0.95, 3.1, 9.8, 31, and 100 mg test item/L) under defined conditions. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations. The concentrations	X

Section A7.4.1.3 Growth inhibition test on algae(03)

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

		were verified with analytical measurements.	
5.2	**Results and discussion**		
5.2.1	NOEC	NOE_yC = 3.1 mg/L: NOE_bC = 3.1 mg/L; NOE_rC = 9.8 mg/L	
5.2.2	EC_{50}	E_yC_{50} = 14.72 mg/L; E_bC_{50} = 17.18 mg/L; E_rC_{50} = 30.62 mg/L	
5.3	**Conclusion**	Validity criteria are summarised in table A7_4_1_3-9. A clear dose – response relationship can be derived from the cell concentration data.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	None	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

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

	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 A7_4_1_3-1: Test organisms

Criteria	Details
Species	Planktonic freshwater algal species *Desmodesmus subspicatus* (former name: *Scenedesmus subspicatus).*
Strain	(Chodat) Hegewald et Schmidt Strain No. 86.81 SAG.
Source	Obtained from "The Collection of Algal Cultures" of the Institute for Plant Physiology, University Goettingen, Germany.
Laboratory culture	Yes
Method of cultivation	The algae are cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines.
Pre-treatment	The cells were taken from an exponentially growing pre-culture, which was set up 3 days prior to the test start under the same conditions as in the test.
Initial cell concentration	Test started with a biomass of 5000 cells per mL nutrient solution

Table A7_4_1_3-2: Test system

Criteria	Details
Volume of culture flasks	50 mL Erlenmeyer flasks covered with glass dishes
Culturing apparatus	Incubation was performed under standardised conditions according to the mentioned guidelines. It was performed in a water bath and under continuous stirring.
Light quality	Continuous illumination at 7507 Lux (mean value), with a maximum deviation of ±15%
Procedure for suspending algae	Continuously stirred by magnetic stirrers
Number of vessels/concentration	Control: 6 flasks; Treatments: 3 flasks
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-3: Test conditions

Criteria	Details
Test temperature	23 ± 2 °C
pH	7.9 at test start 8.1 – 8.4 at test end
Aeration of dilution water	No data
Light intensity	7320-7800 Lux (minimum and maximum value of measurements at 6 places distributed over the experimental area at the surface of the test media)
Photoperiod	Continuous illumination in the incubator (24 h/day)

Table A7_4_1_3-4: Cell ~~numbers~~ density at different test substance concentrations during test (average* values)

Nominal concentration (mg/L)	Average Cell ~~Number~~ Density (initial cell density: 5,000 cells/mL)		
	After 24 h	After 48 h	After 72 h
Control	~~36000~~ **35000**	149000	724000
0.95	36000	145000	~~73000~~ **737000**
3.1	31000	141000	743000
9.8	28000	138000	567000
31	24000	49000	60000
100	-5000	-8000	6000

* Number of samples: control: 6 flasks; each test substance concentration level: 3 vessels

Table A7_4_1_3-5: Growth rates μ and percentage inhibition of μ during the test period and results of the Bonferroni t-Test (α = 0.05, one-sided) with the growth rates μ

Nominal concentration (mg/L)	Growth rates μ [L/day] and % inhibition of μ								
	0-24 h			0-48 h			0-72 h		
	μ	%	t-values	μ	%	t-values	μ	%	t-values
Control	1.955	0.0	-	1.697	0.0	-	1.658	0.0	-
0.95	1.981	-1.3	1.960+	1.683	0.8	-1.176-	1.664	-0.4	0.107-
3.1	1.820	6.9	1.856+	1.670	1.6	-2.257+	1.667	-0.5	0.149-
9.8	1.727	11.7	2.000+	1.660	2.2	-3.074+	1.577	4.9	-1.407-
31	1.566	19.9	2.000+	1.136	33.0	-45.817+	0.823	50.3	-14.498+
100	-6.018	407.8	1.732+	-4.259	350.9	-486.480+	0.038	97.7	-28.130+

-% inhibition: increase in growth relative to that of control
+ mean value significantly different from the control

Table A7_4_1_3-6: Area under the growth curves A and percentage inhibition of A during the test period and results of the Bonferroni t-Test (α = 0.05, one-sided) with the area under the growth curves A

Nominal concentration (mg/L)	Growth rates µ [L/day] and % inhibition of µ								
	0-24 h			0-48 h			0-72 h		
	A	%	t-values	µ	%	t-values	µ	%	t-values
Control	1.52	0.0	-	10.24	0.0	-	53.39	0.0	-
0.95	1.56	-2.9	0.599-	10.12	1.2	-0.512-	53.73	-0.6	0.771-
3.1	1.30	14.5	-2.994+	9.40	8.2	-3.511+	53.08	0.6	-0.692-
9.8	1.16	23.7	-4.910+	8.98	12.4	-5.266+	43.76	18.0	-21.654+
31	0.95	37.6	-7.783+	4.07	60.2	-25.672+	8.99	83.2	-99.883+
100	-0.22	114.5	-23.691+	-0.69	106.7	-45.490+	-0.87	101.6	-122.075+

-% inhibition: increase in growth relative to that of control
+ mean value significantly different from the control

Table A7_4_1_3-7: Yield y and percentage inhibition of y during the test period and results of the Williams Test (α = 0.05, one-sided) with the yield y

Nominal concentration (mg/L)	Yield y [mg/L] and % inhibition of y								
	0-24 h			0-48 h			0-72 h		
	y	%	t-values	y	%	t-values	y	%	t-values
Control	3.04	0.0	-	14.41	0.0	-	71.88	0.0	-
0.95	3.13	-2.9	0.599-	13.99	2.9	-1.325-	73.23	-1.9	1.709-
3.1	2.60	14.5	-2.994+	13.60	5.6	-2.540+	73.76	-2.6	1.709-
9.8	2.32	23.7	-4.910+	13.32	7.6	-3.423+	56.25	21.7	-16.510+
31	1.90	37.6	-7.783+	4.36	69.8	-31.583+	5.48	92.4	-701.144+
100	-0.44	114.5	-23.691+	-0.50	103.5	-46.837+	0.14	99.8	-75.790+

Table A7_4_1_3-8: Summary of the results from a 72 h growth inhibition test with p-chloro-m-cresol and *Desmodesmus subspicatus*

Inhibition-Parameter	Endpoint	Value (mg a.s./L)	95% confidence limits
Yield (72 h)	E_yC_{50}	14.72	14.621 – 14.815
	LOE_yC	9.8	-
	NOE_yC	3.1	-
Biomass (72 h)	E_bC_{50}	17.18	17.001 – 17.367
	LOE_bC	9.8	-
	NOE_bC	3.1	-
Growth Rate (72 h)	E_rC_{50}	30.62	29.319 – 31.981
	LOE_rC	31	-
	NOE_rC	9.8	-

Table A7_4_1_3-9: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥ 80% of initial concentration during test	X	

Criteria for poorly soluble test substances	Not applicable	-

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (01_02)

Annex Point IIA VII.7.4

Official use only

		1 REFERENCE
1.1	**Reference**	Kanne, R. (1988): Preventol CMK – Toxicity towards Bacteria. Bayer AG, WV-LE Umweltschutz, Leverkusen, Germany, Report No. 88105507 (unpublished), Date: 1988-02-10. Weyers, A. (2006): Preventol CMK – Toxicity towards Bacteria. Re-Evaluation based on Study Report No. 88105507, corresponding raw data and additional information provided by the sponsor. Bayer Industry Services, Leverkusen, Germany, Report (unpublished), Date: 2006-06-29.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, ISO regulation 8192 (1986), comparable with OECD Guideline 209
2.2	**GLP**	GLP was not compulsory at time of testing
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	p-chloro-m-cresol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Test substance concentrations are not confirmed by analytical method.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference substance**	Yes, 3,5-Dichlorophenol

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (01_02)

Annex Point IIA VII.7.4

3.3.1	Method of analysis for reference substance	Reference substance concentrations are not confirmed by analytical method	
3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic nutrient medium. For detailed composition of test medium see Re-Evaluation Report, page 8.	X
3.4.2	Inoculum / test organism	See Table A7_4_1_4-1	
3.4.3	Test system	The defined quantity of activated sludge is mixed with synthetic nutrient medium and a respiratory rate is measured after 30 minutes. This rate is compared to those measured in test preparations with various concentrations of the test substance. The inhibitory effect of the test item at a particular concentration is expressed as a percentage of the mean of the respiration rates of two controls. An EC_{50} value is calculated from the respiration rates at different test item concentrations.	
3.4.4	Test conditions	See Table A7_4_1_4-2	
3.4.5	Duration of the test	After an exposure period of 30 minutes, temperature and pH of the exposure medium is measured. Oxygen consumption is measured and recorded after an aeration period of 30 minutes.	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen consumption, temperature and pH of the exposure medium are measured.	
3.4.8	Sampling	After exposure time, the mentioned parameters are measured in the test vessels.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Two controls without test substance are included in the test design, one at the start and the other at the end of the test series. Additionally a physico-chemical oxygen consumption control was included in the test design.	
3.4.11	Statistics	Inhibitory effect of the test item: Percentage of the mean of the respiration rates of two controls. EC_{50} values: Determined by probit analysis	
		4 RESULTS	
4.1	**Preliminary test**	A range-finding test preceded a definitive test. The obtained information about the range of concentrations is used in the main test.	
4.1.1	Concentration	First range-finding test: Test substance concentrations: 100, 1000 and 10000 mg/L; Second range-finding test:	

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (01_02)

Annex Point IIA VII.7.4

		Test substance concentrations: 1, 10 and 100 mg/L;
4.1.2	Effect data	Since the first range-finding test showed an inhibition greater than 50% at 100 mg/l, a second range-finding test with lower test substance concentrations was conducted. The second test revealed inhibition rates of 3 % and 6 % for test substances concentrations of 1 and 10 mg/L, respectively.
4.2	**Results test substance**	See Table A7_4_1_4-3
4.2.1	Initial concentration of test substance	Nominal concentrations: 10, 18, 32, 56 and 100 mg a.i./L
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical methods.
4.2.3	Growth curves	No graph available
4.2.4	Cell concentration data	Not reported
4.2.5	Concentration/ response curve	The dose-effect relationship is shown in a graph (see Re-Evaluation Report, page 21). Effect values have been plotted against the corresponding concentrations on semi-logarithmic paper.
4.2.6	Effect data	EC_{50} = 60.3 mg/L
4.2.7	Other observed effects	-
4.3	**Results of controls**	See Table A7_4_1_4-3
4.4	**Test with reference substance**	Performed with 3,5-Dichlorophenol
4.4.1	Concentrations	1 and 20 mg/L
4.4.2	Results	EC_{50} = 8.5 mg/L; See Table A7_4_1_4-4
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	A study according to ISO 8192 (1986), comparable to OECD Guideline 209 was performed to assess the toxicity of p-Chloro-m-cresol to aquatic bacteria. The activated sludge was exposed to the test substance at different concentrations (10, 18, 32, 56 and 100 mg a.i./L). The respiration rate of each mixture was determined after 30 minutes. EC_{50} values were determined by probit analysis.
5.2	**Results and discussion**	50 % inhibition of respiration was determined at EC_{50} = 60.3 mg/L p-Chloro-m-cresol. The test substance showed 74% respiration inhibition of activated sludge at a concentration of 100 mg a.s./L.
5.2.1	EC_{20}	EC_{25} = 20.4 mg/L

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (01_02)

Annex Point IIA VII.7.4

5.2.2	EC_{50}	60.3 mg/L	
5.2.3	EC_{80}	EC_{75} = 179 mg/L, EC_{90} = 476 mg/L	
5.3	**Conclusion**	The test is considered as valid, since the relevant validity criteria for the applied method are fulfilled.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	No	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

	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 A7_4_1_4-1: Inoculum/Test organism

Criteria	Details
Nature	Activated sludge
Species	Mixed population of aquatic micro-organisms
Strain	-
Source	Laboratory sewage treatment plant (3 L), treating predominantly industrial sewage
Sampling site	Bürrig near Leverkusen/Germany
Laboratory culture	Yes
Method of cultivation	Aeration of the activated sludge at 20 °C ± 2 °C, daily feed with 50 ml of synthetic medium.
Preparation of inoculum for exposure	First, the sludge is settled and supernatant is decanted. After centrifuging (20 min at 4000 rpm and 20°C) the supernatant is decanted again. 1 g of wet sludge is dried in order to calculate the amount of wet sludge to achieve a concentration of activated sludge of 2 g/L (dry wet) suspended solids. The calculated amount of sludge is first dissolved in synthetic medium and then filled up to a defined end volume with de-ionized water.
Pre-treatment	None
Initial cell concentration	Concentration of Inoculum: 2 g/L test substance

Table A7_4_1_4-2: Test conditions

Criteria	Details
Test temperature	20 °C ± 2 °C,
pH	pH values during exposure: 8.5 (test substance), 8.2-8.5 (controls), 7.3 (physico-chemical oxygen consumption control), 8.5-8.6 (reference substance)
Aeration of dilution water	Permanent aeration during incubation time.
Suspended solids concentration	600.00 mg/L suspended solids

Table A7_4_1_4-3: Results for p-Chloro-m-cresol: Respiratory rates, oxygen-consumption and respiration inhibition of the treated activated sludge

Nominal test concentration [mg a.i./L]	Respiratory rate [mg/Lx h]	Phys.-chem. O_2 consumption [mg/Lx h]	Respiratory rate *minus* phys.-chem. O_2 consumption [mg/Lx h]	Inhibition [%]
10	22	0.0*	22	19
18	22	0.0*	22	19
32	18	0.0*	18	34
56	18	0.0*	18	34
100	7	0.0*	7	74
Control 1	26.4			
Control 2	28.0			
Control, mean	27.2			

*: The physico-chemical oxygen consumption has been determined at 100 mg/L and 10000 mg/L test substance concentrations. As no physico-chemical oxygen consumption was observed at that concentration, this observation also holds true for the lower test substance concentrations.

Table A7_4_1_4-4: Results for the reference substance 3,5-Dichlorphenol: Respiratory rates and respiration inhibition of the treated activated sludge

Nominal test concentration [mg a.i./L]	Respiratory rate [mg/L/h]	Inhibition [%]
1	24	12
20	9	68

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (03)

Annex Point IIA VII.7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Neuhahn, A. (2008): Activated Sludge, Respiration Inhibition Test with Preventol CMK Pastillen. Currenta GmbH & Co. OHG, Services Analytik, Leverkusen, Germany, Report No. 2006/0025/16 (unpublished), Date: 2008-08-19.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EC Method C.11: Activated sludge respiration inhibition (2008); OECD Guideline 209 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	p-chloro-m-cresol	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2 of dossier	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility: ca. 4 g/L at 20°C, Vapour pressure: ca. 0.04 mbar at 20°C	X
3.1.6	Method of analysis	Test substance concentrations are not confirmed by analytical method.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes, 3,5-Dichlorophenol	
3.3.1	Method of analysis for reference substance	Reference substance concentrations are not confirmed by analytical method	

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (03)

Annex Point IIA VII.7.4

3.4	**Testing procedure**	The purpose of this test method was mto provide a rapid screening method whereby substances which may adversely affect aerobic microbial treatment plants can be identified.
3.4.1	Culture medium	Synthetic nutrient medium, was made by dissolving the following amounts of substance in 1 litre of water: 16.0 g peptone, 11.0 g meat extract, 3.0 g urea, 0.7 g NaCl, 0.4 g $CaCl_2$ x 2 H_2O, 0.2 g $MgSO_4$ x 7 H_2O, 2.8 g K_2HPO_4.
3.4.2	Inoculum / test organism	See Table A7_4_1_4-1
3.4.3	Test system	The defined quantity of activated sludge (100 mL) is mixed with synthetic nutrient medium (8 mL) and added to the dissolved test item. The mixture was filled up to 250 mL with deionised water and aerated at 20°C ± 2 °C. Oxygen consumption was measured and recorded after an aeration time of 3 hours. The inhibitory effect of the test item at a particular concentration is expressed as a percentage of the mean of the respiration rates of two controls. An EC_{50} value is calculated from the respiration rates at different test item concentrations.
3.4.4	Test conditions	See Table A7_4_1_4-2
3.4.5	Duration of the test	3 hours
3.4.6	Test parameter	Respiration inhibition. The respiration rate for each test substance concentration was determined graphically from the linear part of the curve of oxygen-content versus time.
3.4.7	Analytical parameter	Oxygen consumption, temperature and pH of the exposure medium are measured.
3.4.8	Sampling	After exposure time, the mentioned parameters are measured in the test vessels.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Two controls without test substance are included in the test design, one at the start and the other at the end of the test series. Additionally a physico-chemical oxygen consumption control was included in the test design (prepared as well as the test item, but without activated sludge).
3.4.11	Statistics	Inhibitory effect of the test item: Percentage of the mean of the respiration rates of two controls. EC_{50} values: Determined by probit analysis of the respiration rates at different test substance concentrations.

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (03)

Annex Point IIA VII.7.4

		4 RESULTS	
4.1	**Preliminary test**	A range-finding test preceded a definitive test. The obtained information about the range of concentrations is used in the main test.	
4.1.1	Concentration	First range-finding test: Test substance concentrations: 100, 1000 and 10000 mg/L; Second range-finding test: Test substance concentrations: 1, 10 and 100 mg/L;	X
4.1.2	Effect data	Since the first range-finding test showed an inhibition greater than 50% at 100 mg/l, a second range-finding test with lower test substance concentrations was conducted. The second test revealed inhibition rates of 3 % and 6 % for test substances concentrations of 1 and 10 mg/L, respectively.	X
4.2	**Results test substance**	Results of range-finding test: See attached Tables A7_4_1_4-3 to A7_4_1_4-6. Results of main test: See attached Tables A7_4_1_4-7 to A7_4_1_4-10.	
4.2.1	Initial concentration of test substance	Nominal concentrations: Range-finding test: 10, 100 and 1000 mg a.i./L Main test: 10, 18, 32, 56 and 100 mg a.i./L	
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical methods.	
4.2.3	Growth curves	Results of probit calculation (dose-response curve): See attached Figure A7_4_1_4-1	
4.2.4	Cell concentration data	Not reported	
4.2.5	Concentration/ response curve	The dose-effect relationship is shown in a graph (see attached Figure A7_4_1_4-1). Effect values have been plotted against the corresponding concentrations on semi-logarithmic paper.	
4.2.6	Effect data	EC_{50} = 41.4 mg/L (95 % confidence limits: 30.8 – 58.7), EC_{10} = 5.7 mg/L (95 % confidence limits: 1.6 – 10.2).	
4.2.7	Other observed effects	-	
4.3	**Results of controls**	See attached tables	
4.4	**Test with reference substance**	Performed with 3,5-Dichlorophenol	
4.4.1	Concentrations	Nominal concentrations of 3,5-dichlorophenol: Range-finding test: 5, 10 and 20 mg/L	

Section A7.4.1.4 Inhibition to microbial activity (aquatic) (03)

Annex Point IIA VII.7.4

		Main test: 2.5, 5, 10, 20 and 40 mg/L
4.4.2	Results	EC_{50} = 7.2 mg/L; See attaches Tables for details (A7_4_1_4-3 to A7_4_1_4-10)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A study according to EU Method c.11 (2008) and OECD Guideline 209 1984) was performed to assess the toxicity of p-Chloro-m-cresol to aquatic bacteria. The activated sludge was exposed to the test substance at different concentrations (10, 18, 32, 56 and 100 mg a.i./L). The respiration rate of each mixture was determined after 3 hours. EC_{50} values were determined by probit analysis.
5.2	**Results and discussion**	50 % inhibition of respiration was determined at EC_{50} = 41.4 mg/L p-Chloro-m-cresol. The test substance showed 100 % respiration inhibition of activated sludge at a concentration of 1000 mg a.s./L.
5.2.1	EC_{20}	EC_{10} = 5.7 mg/L; EC_{20} = 11.3 mg/L
5.2.2	EC_{50}	41.4 mg/L
5.2.3	EC_{80}	151.2 mg/L
5.3	**Conclusion**	The test is considered as valid, since the relevant validity criteria for the applied method are fulfilled.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

	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 A7_4_1_4-1: Inoculum/Test organism

Criteria	Details
Nature	Activated sludge
Species	Mixed population of aquatic micro-organisms
Strain	-
Source	Aeration tank of a domestic sewage treatment plant
Sampling site	Cologne-Stammheim/Germany
Laboratory culture	Yes
Method of cultivation	Aeration of the activated sludge at 20 °C ± 2 °C, daily feed with synthetic medium.
Preparation of inoculum for exposure	First, the sludge is settled and supernatant is decanted. After centrifuging (15 min at 4000 rpm and 20°C) the supernatant is decanted again. 1 g of wet sludge is dried in order to calculate the amount of wet sludge to achieve a concentration of activated sludge of 3 g/L (dry wet) suspended solids. The calculated amount of sludge is first dissolved in synthetic medium and then filled up to a defined end volume with de-ionized water.
Pre-treatment	None
Initial cell concentration	Concentration of Inoculum: 3 g/L (dry weight)

Table A7_4_1_4-2: Test conditions

Criteria	Details
Test temperature	20 °C ± 2 °C,
pH	pH values of the suspension before application: 7.4 (range-finding test), 7.1 (main test) For pH values during exposue time refer to attached result tables.
Aeration of dilution water	Permanent aeration during incubation time (3 h).
Suspended solids concentration	Pre-test and main test: 800 mg/L suspended solids

Table A7_4_1_4-3: Range-finding test (test substance CMK): Oxygen content, temperature and pH values during exposure phase

	Test item concen-tration [mg/L]	O_2 start [mg O_2/L]	O_2 end [mg O_2/L]	Time (start-end) [min.]	Temp. [°C]	pH
Test item	10	6.1	3.0	9	20.7	7.9
	100	6.9	5.9	9	20.8	7.7
	1000	7.8	7.8	9	20.9	7.8
Control 1		6.2	2.7	8	20.6	7.9
Control 2		5.9	2.5	8	20.7	7.8
Physico-chemical oxygen consumption control	1000	7.5	7.5	9	20.8	7.3

Table A7_4_1_4-4: Range-finding test (reference substance): Oxygen content, temperature and pH values during exposure phase

	Test item concen-tration [mg/L]	O_2 start [mg O_2/L]	O_2 end [mg O_2/L]	Time (start-end) [min.]	Temp. [°C]	pH
3,5-Dichloro-phenol	5	6.3	3.4	8	20.4	7.9
	10	6.3	4.9	9	20.6	7.7
	20	7.0	6.5	8	20.6	7.8

Table A7_4_1_4-5: Range-finding test (test substance CMK): Respiratory rates, oxygen-consumption* and respiration inhibition of the treated activated sludge

Test item concentration (nominal) [mg/L]	Respiratory rate test item [mg/L · h]	Phys.-chem. O_2 consumption [mg/L · h]	Respiratory rate - phys.-chem. O_2 consumption [mg/L · h]	Inhibition [%]
10	20.7	0.0*	20.7	20.1
100	6.7	0.0*	6.7	74.2
1000	0.0	0.0	0.0	100.0
Control. mean	25.9			
Control 1	26.3			
Control 2	25.5			

*: The physico-chemical oxygen consumption has been determined at 1000 mg/L test substance concentrations. As no physico-chemical oxygen consumption was observed at that concentration, this observation also holds true for the lower test substance concentrations.

Table A7_4_1_4-6: Range-finding test (reference substance): Respiratory rates and respiration inhibition of the treated activated sludge

Reference compound concentration (nominal) [mg/L]	Respiratory rate test item [mg/L · h]	Inhibition [%]
5	21.8	15.9
10	9.3	63.9
20	3.8	85.5
Control. mean	25.9	
Control 1	26.3	
Control 2	25.5	

Table A7_4_1_4-7: Main test (test substance CMK): Oxygen content, temperature and pH values during exposure phase

	Test item concen-tration [mg/L]	O_2 start [mg O_2/L]	O_2 end [mg O_2/L]	Time (start-end) [min.]	Temp. [°C]	pH
Test item	10	5.7	2.6	7	20.6	7.7
	18	5.5	2.8	7	20.5	7.7
	32	5.8	2.7	9	20.7	7.7
	56	5.9	3.9	8	20.8	7.6
	100	6.2	5.0	9	20.9	7.5
Control 1		5.7	2.8	5	21.1	7.7
Control 2		5.4	2.7	5	20.6	7.7
Physico-chemical oxygen consumption control *		7.5	7.5	9	20.8	7.3

Table A7_4_1_4-8: Main test (reference substance): Oxygen content, temperature and pH values during exposure phase

	Test item concen-tration [mg/L]	O_2 start [mg O_2/L]	O_2 end [mg O_2/L]	Time (start-end) [min.]	Temp. [°C]	pH
3,5-Dichloro-phenol	2.5	5.9	2.8	7	20.8	7.8
	5	5.2	2.6	6	20.9	7.6
	10	6.5	5.0	9	20.8	7.5
	20	7.0	6.6	8	20.6	7.5
	40	7.0	6.8	8	20.5	7.6

Table A7_4_1_4-9: Main test (test substance CMK): Respiratory rates, oxygen-consumption* and respiration inhibition of the treated activated sludge

Test item concentration (nominal) [mg/L]	Respiratory rate test item [mg/L · h]	Phys.-chem. O_2 consumption [mg/L · h]	Respiratory rate - phys.-chem. O_2 consumption [mg/L · h]	Inhibition [%]
10	26.6	0.0*	26.6	20.9
18	23.1	0.0*	23.1	31.1
32	20.7	0.0*	20.7	38.5
56	15.0	0.0*	15.0	55.4
100	8.0	0.0	8.0	76.2
Control. mean	33.6			
Control 1	34.8			
Control 2	32.4			

*: The physico-chemical oxygen consumption has been determined at 1000 mg/L test substance concentrations. As no physico-chemical oxygen consumption was observed at that concentration, this observation also holds true for the lower test substance concentrations.

Table A7_4_1_4-10: Main test (reference substance): Respiratory rates, oxygen-consumption and respiration inhibition of the treated activated sludge

Reference compound concentration (nominal) [mg/L]	Respiratory rate test item [mg/L · h]	Inhibition [%]
2.5	26.6	20.9
5	26.0	22.6
10	10.0	70.2
20	3.0	91.1
40	1.5	95.5
Control. mean	33.6	
Control 1	34.8	
Control 2	32.4	

Figure A7_4_1_4-1: Main test (test substance CMK): Results of the probit analysis

Results of the probit analysis

Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits

Parameter	EC10	EC20	EC50	EC80
Value [mg/l]	5.7	11.3	41.4	151.2
lower 95%-cl	1.6	4.8	30.8	93.5
upper 95%-cl	10.2	17.1	58.7	421.7
lower 99%-cl	0.7	2.9	23.5	49.9
upper 99%-cl	22.1	29.0	76.9	791.0

n.d.: not determined due to mathematical reasons

Computation of variances and confidence limits was adjusted to metric data (Christensen & Nyholm 1984). Inhibitions lower equal 0% or greater equal 100% were replaced by 0.100 and 99.900. respectively. Slope function after Litchfield and Wilcoxon: 4.687

(The slope function is derived from the slope. b. of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

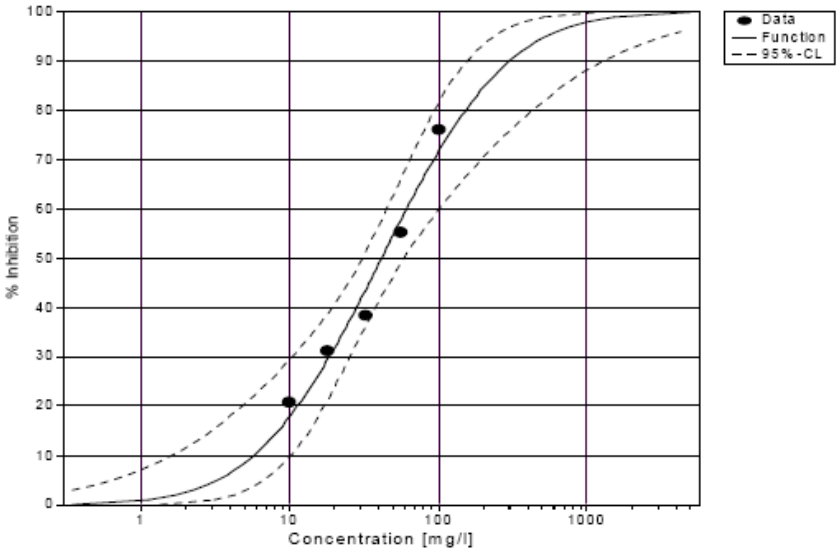

Figure 1: EC_{50}-determination of Preventol CMK Pastillen.

Section A7.4.2 (01) Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5

Official use only

		1 REFERENCE
1.1	**Reference**	Paul, A. (2007): p-Chloro-m-cresol (CMK) - Calculation of the Bioconcentration Factor (BCF). Dr. Knoell Consult GmbH, Leverkusen, Germany, report no. KC-BCF-07/07, date: 2007-05-31 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not applicable, calculation method
2.2	**GLP**	Not applicable
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	p-Chloro-m-cresol (CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Not applicable
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Log Kow = 3.02 (Ref.: Reusche, 1991)
3.1.5	Radiolabelling	--
3.1.6	Method of analysis	--
3.2	**Reference substance**	--
3.2.1	Method of analysis for reference substance	-
3.3	**Testing/estimation procedure**	
3.3.1	Test system/ performance	Not applicable
3.3.2	Estimation of bioconcentration	The bioconcentration factor in aquatic organisms (fish) was calculated using the equation 74 of the Technical Guidance Document on Risk Assessment (EU, 2003). The bioconcentration factor (BCF) can be measured experimentally directly. A number of test guidelines are available for the direct measurement of bioconcentration, of which OECD 305 is the most widely applied. The assessment of the BCF is necessary for chemicals which are, based on base-set data, considered to have a log Kow greater

Section A7.4.2 (01) Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5

than 3.

Another possibility is the estimation of BCF from log Kow. A linear model is recommended for log Kow up to 6 and a non-linear model for log Kow values from 6 to 10.

Linear equation log Kow < 6

$$\log BCF_{fish} = 0.85 \times \log Kow - 0.70 \text{; n=55, } r^2\text{=0.90}$$

n is the number of data
r^2 correlation coefficient
Kow: octanol-water partition coefficient [-]
BCF_{fish}: bioconcentration factor for fish on wet weight basis [l*kg wet fish]

This relationship applies to compounds with a MW less than 700.

The linear model generated by Veith et al. (1979) is based on BCF data for fathead minnows (*Pimephales promelas*). Log Kow is used as descriptor variable. This model has been validated externally in the past, using BCF data for 267 substances (Devillers et al., 1995). The root mean square error of the predictions was 0.58 for log Kow < 6.

This model can be used to derive estimates for neutral, non-polar and non-ionised chemicals. These types of chemicals are usually biotransformed relatively slowly. They are not applicable to ionic substances, partly ionised chemicals and organometallics.

4 RESULTS

4.1	**Experimental data**	
4.1.1	Mortality/behaviour	--
4.1.2	Lipid content	--
4.1.3	Concentrations of test material during test	--
4.1.4	Bioconcentration factor (BCF)	Bioconcentration factor is not based on measurements
4.1.5	Uptake and depuration rate constants	--
4.1.6	Depuration time	--
4.1.7	Metabolites	--
4.1.8	Other Observations	--
4.2	**Estimation of bioconcentration**	The obtained BCF by this method is 73.6.

Section A7.4.2 (01) Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The BCF of CMK in fish was estimated using the QSAR-approach as recommended in the Technical Guidance Document on Risk Assessment (EU, 2003) based on a measured log Kow value. If measured BCF values are not available, the BCF for fish can be predicted from the relationship between Kow and BCF. Various methods are available to calculate Kow. For substances with a log Kow of between 2 and 6 the following linear relationship can be used as developed by Veith et al. (1979): $\log BCF_{fish} = 0.85 \times \log Kow - 0.70$
5.2	Results and discussion	Considering a log Kow-value of 3.02 which was obtained in a previously performed experimental study, the calculated BCF-value of CMK was 73.6.
5.3	**Conclusion**	Based on a log Kow value of 3.02, obtained from an experimental study, a BCF of 73.6 is obtained. This value indicates a low bioaccumulation potential of CMK in aquatic organisms.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	--

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.2 (02) Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Cyprinus carpio*

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		1 REFERENCE
1.1	**Reference**	MITI (Ministry of International Trade & Industr) (1992): Biodegradation and bioaccumulation: Data of existing chemicals based on the CSCL Japan. Published by Japan Chemical Industry Ecology-Toxicology & Information Center, 1992
1.2	**Data protection**	No
1.2.1	Data owner	-
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	-
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, According to "Bioaccumulation test of chemical substance in fish and shellfish", stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974. Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No. 1). This guideline corresponds to OECD Guidelines for Testing of Chemicals, No. 305C, Bioaccumulation: Degree of Bioconcentration in Fish" (May 12, 1981).
2.2	**GLP**	Not mentioned in the publication
2.3	**Deviations**	Not mentioned in the publication
		3 MATERIALS AND METHODS
3.1	**Test material**	4-Chloro-m-cresol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Not mentioned in the publication
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	-
3.1.5	Radiolabelling	No
3.1.6	Method of analysis	Not mentioned in the publication
3.2	**Reference substance**	No
3.2.1	Method of analysis for reference substance	-
3.3	**Testing/estimation procedure**	

Section A7.4.2 (02) Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Cyprinus carpio*

3.3.1	Test system/ performance	Test animals The carp (*Cyprinus carpio*) used in this study were obtained from Sugishima fish farm (Japan). Fish were fed with pelleted feed for carp (manufacturer: Japan Haigo Shiryo K.K.) at amounts corresponding to about 2% of the total body weight of test fish twice a day by halves. On the day of fish sampling feed was not supplied. Test system 1-2 months after external disinfection which was carried out according to static condition for 24 h in an aqueous solution containing 50 mg/L of Terramycin (Taito Pfizer) and 7 g/L sodium chloride, fish were reared for acclimation in an acclimation tank according to flow through system at temperature of 25 ± 2 °C for about 28 days. During the period, abnormal fish were removed. Then the fish were transferred to test tanks and reared again for about one month. 15-20 fish per concentration were exposed to Preventol CMK in 100 L glass tanks for 6-8 weeks. The temperature controlled test water was supplied continuously into the flow through system (Aquatron) at a rate of 200-800 mL/min at 25 ± 2 °C. The concentration of dissolved oxygen in the test tank was 6-8 mg/L. At the initiation of exposure, fish weighted about 30 g and the length was about 10 cm. Preparation of the test substance It is necessary to prepare a solution of the test substance 100 times highly concentrated than that in the aquarium. If the test compound is not soluble enough in water, suitable solubilisers were used. Sampling Fish: During the uptake phase test fish analysis were taken every two weeks. Control fish were analysed before the initiation and the termination of exposure. Water: Water samples were analysed twice a week.	X
3.3.2	Estimation of bioconcentration	The bioconcentration factor (BCF) was determined by the following calculation: $BCF = \frac{\text{Concentration of test substance in fish}}{\text{Concentration of test substance in water}}$	
		4 RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/behaviour	Not mentioned in the publication	X
4.1.2	Lipid content	Lipid content at start exposure: 2-6%	X

Section A7.4.2 (02) Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Cyprinus carpio*

4.1.3	Concentrations of test material during test	Nominal: 2 mg/L, 20 mg/L	X
4.1.4	Bioconcentration factor (BCF)	5.5 – 11 at 2 mg/L 6.7 – 13 at 20 mg/L	X
4.1.5	Uptake and depuration rate constants	Not mentioned in the publication	
4.1.6	Depuration time	Not mentioned in the publication	
4.1.7	Metabolites	Not mentioned in the publication	
4.1.8	Other Observations	Not mentioned in the publication	
4.2	**Estimation of bioconcentration**	The calculated Bioconcentration factor is based on measurements.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The potential for Bioconcentration of 4-Chloro-m-cresol was investigated in a flow-through fish test according to OECD 305C, "Bioaccumulation: Degree of Biocencentration in Fish" (May 12, 1981). The test was performed with test substance concentration levels of 2 mg/L and 20 mg/L.	X
5.2	**Results and discussion**	BCF was determined to be 5.5 – 11 and 6.7 - 13 at concentrations of 2 mg/L and 20 mg/L. The results clearly demonstrate, that 4-Chloro-m-cresol does not have a potential for bioaccumulation.	X
5.3	**Conclusion**	The results clearly demonstrate, that Preventol CMK does not have a potential for bioaccumulation.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	- Characterisation of test substance (Batch-No., Purity) is missing - Insufficient description of test system and test procedure - Results are not summarised accurately.	

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Cyprinus carpio*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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*

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Cyprinus carpio*

Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.4.3.1 Prolonged toxicity to an appropriate species of fish (01_02)
Annex Point IIIA XIII 2.2

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		1 REFERENCE
1.1	**Reference**	[redacted] (1991): Preventol CMK: Prolonged Toxicity Test with Zebrafish (*Brachydanio rerio*). [redacted] 1991-11-13. [redacted] (2006): Preventol CMK – Fish, Prolonged Toxicity Test. Re-Evaluation based on Study Report 212 A/90 FL, Corresponding Raw Data and Additional Information Provided by the Sponsor. [redacted] Date: 2006-07-05.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes; UBA-Draft method: "Prolonged Toxicity Test with Zebrafish - *Brachydanio rerio*" (1984); this guideline is comparable to OECD Guideline 204 "Fish, Prolonged Toxicity Test: 14-day Study" (adopted 1984)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	4-chloro-3-methylphenol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference substance**	None
3.3.1	Method of analysis	-

Section 7.4.3.1 Prolonged toxicity to an appropriate species of fish (01_02)
Annex Point IIIA XIII 2.2

	for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_2-1	
3.4.2	Test organisms	See table A7_4_3_2-2	
3.4.3	Handling of embryos and larvae (OECD 210/212)	Not applicable, adult fish were used for the test, reproduction was not a test parameter	
3.4.4	Test system	See table A7_4_3_2-3	
3.4.5	Test conditions	See table A7_4_3_2-4	
3.4.6	Duration of the test	Experimental Phase: 14 days	
3.4.7	Test parameter(s)	Mortality Sub-lethal and behavioural responses (observations) Chemical and physical parameters: Temperature, dissolved oxygen, pH-value, total hardness, acid	
3.4.8	Examination / Sampling	Mortality, incidence of sub-lethal effects as well as behavioural responses were recorded every 24 hours; Fish size (body length): was measured at day 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; Wet body weight: measured at end of exposure (after 14 days); pH values, temperature and dissolved oxygen concentrations were measured at the beginning of the test and every 24 hours thereafter.	
3.4.9	Monitoring of TS concentration	Yes, at study days 0, 2,7,12, 14. The method for determination of test substance in aqueous solutions is described in the test report (pp. 15-17).	X
3.4.10	Statistics	Not reported	
		4 RESULTS	
4.1	**Range finding test**		
4.1.1	Concentrations	No range finding test was carried out for this study.	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentration levels: 0.01, 0.032, 0.1, 0.32, 1.0 and 3.2 mg test substance/L. Additionally an untreated control was run in parallel.	
4.2.2	Actual	Mean measured concentrations:	

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	concentrations of test substance	< 0.014, 0.017, 0.076, 0.306, 1.044, 3.35 mg/L For details see table A7_4_3_2-5.	
4.2.3	Effect data	NOEC (14 days) = 1.0 mg/L, LOEC (14 days) = 3.2 mg/L. Detailed results are summarised in table A7_4_3_2-6 (Mortality and behavioural observations) and table A7_4_3_2-7 (Body length and wet eight of fish).	X
4.2.4	Concentration / response curve	Not included in the report	
4.2.5	Other effects	--	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were visible	
4.3.2	Nature of adverse effects	No adverse effects were visible	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was performed to assess the prolonged toxicity (14 days) of p-Chloro-m-cresol to Zebrafish (*Brachydanio rerio*) under flow-through conditions. The test was performed according to UBA-Draft method: "Prolonged Toxicity Test with Zebrafish -*Brachydanio rerio*" (1984); this guideline is comparable to OECD Guideline 204 "Fish, Prolonged Toxicity Test: 14-day Study" (adopted 1984). p-Chloro-m-cresol was applied at nominal concentration levels of 0.01, 0.032, 0.10, 0.32, 1.0 and 3.2 mg a.i./L. Additionally control experiments were done.	
5.2	**Results and discussion**	Based on the findings stated above the NOEC (14 d) was determined as 1.0 mg test substance/L and the LOEC (14 d) was 3.2 mg test substance/L.	X
5.2.1	NOEC	1.0 mg/L	
5.2.2	LOEC	3.2 mg/L	X
5.3	**Conclusion**	Based on the findings stated above the NOEC (14 d) was determined as 1.0 mg test substance/L. The validity criteria can be considered as fulfilled. The validity criteria are summarised in table A7_4_3_2-8.	
5.3.1	Other Conclusions	--	

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5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
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 A7_4_3_2-1: Dilution water

Criteria	Details
Source	Synthetic freshwater (according to ISO 7346)
Alkalinity ($CaCO_3$)	At test start, the concentration of the major ions Ca and Mg was 89 mg/L and 15 mg/L, respectively. The acidity (to pH 4.3) was 0.80 mmol/L. During the test, concentrations of Ca and Mg were 91 mg/L and 14 mg/L, respectively. The acidity (to pH 4.3) was 0.77 mmol/L
Hardness ($CaCO_3$)	At test start, hardness of dilution water was 15.3 °dH (275.4 mg/L $CaCO_3$); during the test a value of 15 °dH (270.0 mg/L $CaCO_3$) was detected.
pH	7.0 - 7.5
Oxygen content	8.5 - 9.3 mg/L
Conductivity	Not stated
TOC Content	No
Holding water different from dilution water	No

Table A7_4_3_2-2: Test organisms

Criteria	Details
Species	Zebra fish (*Danio rerio*, formerly *Brachydanio rerio*)
Source	[redacted]
Wild caught	No
Age/size	Day of birth: 04-04-1991 Date of receipt: 27-06-1991 Body length: 2.5-3.5 cm
Kind of food	Tetra Min fish food, ground
Amount of food	Sufficient quantity
Feeding frequency	At least once a day
Post-hatch larvae exposure	Not applicable
Time to first feeding	Not applicable
Feeding of animals during test	Yes
Treatment for disease within 2 weeks proceeding test	Not applicable

Table A7_4_3_2-3: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Renewal of test solution about six times per day
Volume of test vessels	Aerated fish tanks (300 x 135 x 200 mm) containing 5 litres of test medium
Volume/animal	0.5 L
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-4: Test conditions

Criteria	Details
Test temperature	In the range of 20 to 24°C
Dissolved oxygen	Control: 8.5-9.3 mg/L, at 3.2 mg/L test level: 8.5-8.7 mg/L, at 1 mg/L test level: 8.5-9.4 mg/L, at 0.32 mg/L test level:8.5-9.4 mg/L, at 0.1 mg/L test level: 8.1-9.2 mg/L, at 0.032 mg/L test level:8.1-9.5 mg/L, at 0.01 mg/L test level: 8.4-9.2 mg/L,
pH	Control: 7.0-7.5, at 3.2 mg/L test level: 7.5-7.7, at 1 mg/L test level: 7.2-7.7, at 0.32 mg/L test level:7.2-7.8, at 0.1 mg/L test level: 7.1-7.7, at 0.032 mg/L test level:7.1-7.7, at 0.01 mg/L test level: 7.1-7.7
Adjustment of pH	Not stated
Aeration of dilution water	Yes
Intensity of irradiation	Not stated
Photoperiod	16 h light : 8 h dark

Table A7_4_3_2-5 Mean measured concentrations of p-Chloro-m-cresol in the test media

Study Day	Control	Nominal Concentration (mg/L)					
		0.01	**0.032**	**0.10**	**0.32**	**1.0**	**3.2**
0	ND*	< 0.002	0.031	0.1	0.43	1.38	3.84
2	ND	0.06	0.023	0.07	0.28	0.95	2.85
7	ND	< 0.002	0.006	0.07	0.19	0.85	-
12	ND	< 0.002	0.006	0.06	0.26	1.01	-
14	ND	< 0.002	0.020	0.08	0.37	1.03	-
Mean	ND	< 0.014	0.017	0.076	0.306	1.044	3.35
SD	ND	NA**	0.011	0.015	0.095	0.2	0.7
Mean measured in % of nominal concentration			**53.1**	**76.0**	**95.6**	**104.4**	**104.7**

ND* = Not detectable (< 2 µg/L)
NA** = Not applicable

Table A7_4_3_2-6 Cumulative mortality and behavioural observations (Results for Control: Mortality = 0; no visible effects)

Day	Nominal Concentration [g/L]											
	0.01		0.032		0.10		0.32		1.0		3.2	
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
0	0	N	0	N	0	N	0	N	0	N	0	A, B, D, F
1	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	8	A, B, D, F
2	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	8	A, C, D, G
3	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	9	A, C, D, G
4	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	9	E
5	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	10	--
6	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
7	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
8	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
9	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
10	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
11	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
12	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
13	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--
14	0	**N**	0	**N**	0	**N**	0	**N**	0	**N**	--	--

Abbreviations of behavioural observations

N Normal
A Uncontrolled swimming action
B Gasping for air; irregular breathing
C Abnormal swimming action
D Uncontrolled collision with aquarium walls
E Motionless, uncontrolled swimming action when touched
F No fed intake
G Reduced fed intake

Table A7_4_3_2-7 Total wet weight and body length of test fish at the end of the study

Day	Body length [cm] at various test concentrations [mg/L]						
	Control	**0.01**	**0.032**	**0.10**	**0.32**	**1.0**	**3.2**
1	3.3	3.3	3.0	3.3	3.3	3.1	2.8
2	3.0	3.0	2.8	3.2	3.0	3.0	3.0
3	3.1	3.5	3.2	3.4	3.2	3.0	3.0
4	2.7	3.5	3.1	3.5	3.1	3.2	3.1
5	3.5	3.2	3.4	3.2	3.0	3.0	3.0
6	3.2	3.2	3.4	3.1	3.1	3.1	2.9
7	3.0	3.2	3.0	3.0	3.2	3.1	3.1
8	3.0	3.3	2.9	3.5	3.3	3.3	3.0
9	3.1	3.3	3.4	2.9	3.0	3.5	3.0
10	3.1	2.9	3.1	3.2	2.7	2.8	2.7
	Body weight of 10 fish [g]						
14	2.81	2.58	2.78	2.84	3.07	3.10	2.94

Table A7_4_3_2-8 Validity criteria for fish tests according to OECD Guidelines 204

	fulfilled	**Not fulfilled**
Concentration of dissolved oxygen > 60% saturation throughout the test	**yes**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**yes**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**Not applicable**	
Test substance concentrations maintained within ± 20% of mean measured values	**Yes, at NOEC ~~and LOEC concentrations~~ (≥ 0.32 mg/L)**	
No effect on survival nor any other adverse effect found in solvent control	**yes**	
Further criteria for poorly soluble test substances	**Not applicable**	

Section 7.4.3.2
Annex Point IIIA XIII 2.2

Effects on reproduction and growth rate on an appropriate species of fish

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted]: Toxicity of 4-Chloro-3-methylphenol (Preventol CMK) to Rainbow trout (*Oncorhynchus mykiss)* in a prolonged semi static test over 28 days. [redacted] date: 2007-03-28.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes; OECD Guideline for Testing of Chemicals, Section 2, No. 204: "Fish, Prolonged Toxicity Test: 14-day Study", adopted April 04, 1984 OECD Guideline for Testing of Chemicals, Section 2, No. 215: "Fish, Juvenile Growth Test", adopted January 21, 2000
2.2	**GLP**	Yes
2.3	**Deviations**	During the test, the pH was within a range of ± 0.9 pH units instead of ± 0.5
		3 METHOD
3.1	**Test material**	4-Chloro-3-methylphenol (Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	HPLC "Identity and assay of Preventol CMK (Pellets)"; Bayer Industries Services GmbH & Co. OHG; File No. 2006/0025/01
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable, since water solubility of Preventol CMK is about 4 g/L at 20°C
3.3	**Reference substance**	None
3.3.1	Method of analysis for reference	-

Section 7.4.3.2 Annex Point IIIA XIII 2.2 — Effects on reproduction and growth rate on an appropriate species of fish

	substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_2-2	
3.4.2	Test organisms	See table A7_4_3_2-3	
3.4.3	Handling of embryos and larvae (OECD 210/212)	-	
3.4.4	Test system	See table A7_4_3_2-4	
3.4.5	Test conditions	See table A7_4_3_2-5	
3.4.6	Duration of the test	28 days	
3.4.7	Test parameter(s)	Biological parameters: Mortality and signs of intoxication, weighing of test fish Chemical and physical parameters: Measurement of pH-values, dissolved oxygen, water temperature and behaviour of the test item in test water	
3.4.8	Examination / Sampling	Mortality and signs of intoxication: all test fish were observed at least once each working day Weighing of test fish: A subsample of 10 fish from the test fish batch was weighed before the test in order to estimate the mean weight. At the start of the test the individual wet weight of fish in each test vessel was recorded. The individual wet weight of the introduced fish did not exceed 25% of the arithmetic mean weight of the fish batch. Additionally, at the end of the test on day 28 the fish were weighed again. Measurement of pH, dissolved oxygen and water temperature: These parameters in the freshly prepared and old test media of all test concentrations and the control were measured at each test medium renewal. Behaviour of the test item in test water: This parameter was determined at each test medium renewal in the freshly prepared and old test media of all test concentrations.	
3.4.9	Monitoring of TS concentration	Yes, at study days 0, 2, 9, 12, 19, 21, 23 and 26	X
3.4.10	Statistics	The NOEC and the LOEC for the pseudo specific growth rate were evaluated by the Dunnett's multiple t-Test after testing the data for normal distribution and homogeneity of variance using Kolmogorov-Smirnov-test and Bartlett's test, respectively. The software used was ToxRat Professional, Version 2.09, ToxRat® Solutions GmbH, 2005.	
		4 RESULTS	
4.1	**Range finding test**		
4.1.1	Concentrations	Concentrations of up to maximal 100 mg test item/L were tested.	

Section 7.4.3.2 Annex Point IIIA XIII 2.2 — Effects on reproduction and growth rate on an appropriate species of fish

4.1.2	Number/ percentage of animals showing adverse effects	Not mentioned in the report.
4.1.3	Nature of adverse effects	Not mentioned in the report.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentration levels: 0.019, 0.061, 0.20, 0.63 and 2.0 mg test substance/L
4.2.2	Actual concentrations of test substance	Preventol CMK was applied at measured concentration levels of 0.012, 0.044, 0.15, 0.50 and 1.9 mg test substance/L. Additionally a control experiment was conducted. For details see table A7_4_3_2-6.
4.2.3	Effect data	See table A7_4_3_2-7. Mortality and symptoms of intoxication: At the highest test concentration of 1.9 mg test item/L all fish were dead after 6 days, before they died the fish showed several signs of intoxication. No mortality was observed during the test in the test concentration of 0.50 mg test item/L. However, the fish showed reduced feeding, strong ventilation, strongly extended gills and the fish swam mainly on the water surface from day 7 until the end of the test. Furthermore, in the control and in the test concentrations of 0.012, 0.044 and 0.15 mg test item/L one fish died during the test, however the surviving fish showed no signs of intoxication and therefore the death could not be caused by the test item. Additionally, in the test concentration of 0.15 mg test item/L, a second fish died due to attacks of another fish in this concentration. Growth parameters: The mean body weight increased 150% in the control. At 0.012, 0.044 and 0.15 mg test item/L the mean body weight increase ranged between 130% and 146%. At 0.50 mg test item/L the mean body weight increased 65%. Pseudo specific growth rate: The mean pseudo specific growth rate for the control was determined to be 1.40. According to the results of the Dunnett's multiple t-test (one sided smaller, $\alpha = 0.05$) the pseudo specific growth did not differ between the control and the test concentration of 0.012, 0.044 and 0.15 mg test item/L. In contrast, the pseudo specific growth rate decreased significantly in the test concentration of 0.50 mg test item/L compared to the control (0.77). $NOEC_{pseudo\ specific\ growth\ rate}$ = 0.15 mg test item/L $LOEC_{pseudo\ specific\ growth\ rate}$ = 0.50 mg test item/L LLC = 1.9 mg test item/L
4.2.4	Concentration / response curve	Not included in report
4.2.5	Other effects	-

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4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	One fish / 10%
4.3.2	Nature of adverse effects	Fish was dead.
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test was performed according to OECD Guideline 204 (April 04, 1984) and 215 (January 21, 2000). Juvenile Rainbow trout (*Oncorhynchus mykiss*) were exposed in a semi static test to aqueous test media containing Preventol CMK at various concentrations under defined conditions for 28 days. The recorded effects were mortality, symptoms of intoxication and, at the start and the end of the test, the growth parameters body weight and length of surviving fish.
5.2	**Results and discussion**	
5.2.1	NOEC	0.15 mg test substance/L (28 days, pseudo specific growth rate)
5.2.2	LOEC	0.50 mg test substance/L (28 days, pseudo specific growth rate)
5.3	**Conclusion**	For Rainbow trout, the highest concentration tested without toxic effects (NOEC) of the test item Preventol CMK on the growth rate after an exposure period of 28 days was determined to be 0.15 mg test item/L. The lowest concentration tested with toxic effects (LOEC) on the growth rate was determined to be 0.50 mg test item/L due to clearly observed intoxication symptoms at several fish and the statistically significantly reduced growth of the test fish at this test concentration. The lowest lethal concentration (LLC) was 1.9 mg test item/L. The validity criteria can be considered as fulfilled. The validity criteria are summarised in table A7_4_3_2-8.
5.3.1	Other Conclusions	
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
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 A7_4_3_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	No
Other procedures	-

Table A7_4_3_2-2: Dilution water

Criteria	Details
Source	De-ionised water
Alkalinity ($CaCO_3$)	0.8 mmol/L
Hardness ($CaCO_3$)	2.5 mmol/L (= 250.0 mg/L);
pH	6.5 – 8.5
Oxygen content	> 60% of air saturation value (dissolved oxygen concentration)
Conductivity	< 5 µS/cm
TOC Content	Not mentioned in the report
Holding water different from dilution water	No

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	Juvenile males and females; mean body weight: 0.78 ± 0.09 g, mean body length: 4.18 ± 0.12 cm
Kind of food	Commercial feed for Rainbow trout
Amount of food	During holding until one day before test start: maximum ration of 2% body weight per day During the test: daily 4% dry weight based on the mean initial fish wet weight in each test vessel
Feeding frequency	During holding: one times per day during the test: two times per day
Post-hatch larvae exposure	-
Time to first feeding	-
Feeding of animals during test	Yes
Treatment for disease within 2 weeks proceeding test	No

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	Three times per week
Volume of test vessels	24 L glass aquaria
Volume/animal	22 L test medium/10 fish
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	Details
Test temperature	15 - 16 °C
Dissolved oxygen	At least 8.4 mg/L or higher
pH	7.1 – 8.0
Adjustment of pH	No
Aeration of dilution water	Yes Test media were slightly aerated during the test period.
Intensity of irradiation	Light intensity: 320 – 850 lux
Photoperiod	The test chambers were positioned under regulated lighting to produce an overall photoperiod of 16 h light and 8 h dark.

Table A7_4_3_2-6: Mean measured concentrations of Preventol CMK in the test media

Nominal concentration [mg/L]	Mean measured concentration of nominal (%)	Mean measured concentration [mg/L]
Control	n.a.	n.a.
0.019	63 ± 63 *	0.012
0.061	72 ± 49	0.044
0.20	75 ± 35	0.15
0.63	80 ± 21	0.50
2.0	95 ± 4	1.90

* Relative Standard Deviation (RSD)

Table A7_4_3_2-7: Effects of Preventol CMK on Rainbow trout (*Oncorhynchus mykiss*)

Day [1]	Control	Mean measured Preventol CMK concentration [mg/L]				
		0.012	0.044	0.15	0.50	1.9
Mortality and symptoms of intoxication (number of dead fish / number of fish with intoxication symptoms [2] and observed symptoms of intoxication)						
0 (after 2 h)	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 10 (TS, SR, DC)
1	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	7 / 10 (TS)
2	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	7 / 10 (SR, TS)
5	0 / 0	0 / 0	0 / 0	1 / 1	0 / 0	9 / 10 (SR, AP, KR)
6	0 / 0	0 / 0	0 / 0	1 / 1	0 / 0	10 / 10
7	0 / 0	0 / 0	0 / 0	1 / 1	0 / 10 (OB, SV)	10 / 10

Day [1]	Control	Mean measured Preventol CMK concentration [mg/L]				
		0.012	**0.044**	**0.15**	**0.50**	**1.9**
8	0 / 0	0 / 0	0 / 0	1 / 1	0 / 10 (OB, SV, AK)	10 / 10
9	0 / 0	0 / 0	0 / 0	1 / 1	0 / 10 (OB, SV, AK)	10 / 10
12	0 / 0	0 / 0	0 / 0	1 / 1	0 / 10 (OB, SV, AK)	10 / 10
13	0 / 0	0 / 0	0 / 0	1 / 1	0 / 10 (OB, SV, AK)	10 / 10
14	0 / 0	0 / 0	0 / 0	1 / 1	0 / 10 (OB, SV, AK)	10 / 10
15	0 / 0	0 / 0	1 / 1	1 / 1	0 / 10 (OB, SV, AK)	10 / 10
16	0 / 0	0 / 0	1 / 1	2 / 2 [3]	0 / 0	10 / 10
19	1 / 1	1 / 1	1 / 1	2 / 2	0 / 5 (OB, SV)	10 / 10
20	1 / 1	1 / 1	1 / 1	2 / 2	0 / 3 (OB)	10 / 10
21	1 / 1	1 / 1	1 / 1	2 / 2	0 / 4 (OB)	10 / 10
22	1 / 1	1 / 1	1 / 1	2 / 2	0 / 2 (OB)	10 / 10
23	1 / 1	1 / 1	1 / 1	2 / 2	0 / 0	10 / 10
26	1 / 1	1 / 1	1 / 1	2 / 2	0 / 8 (OB)	10 / 10
27	1 / 1	1 / 1	1 / 1	2 / 2	0 / 2 (OB)	10 / 10
28	1 / 1	1 / 1	1 / 1	2 / 2	0 / 0	10 / 10
Body total weight [g]						
At start of the test (mean ± SD)	0.86 ± 0.09	0.86 ± 0.05	0.88 ± 0.05	0.85 ± 0.07	0.86 ± 0.08	0.85 ± 0.08
At end of the test (mean ± SD)	2.15 ± 0.46	1.98 ± 0.28	2.01 ± 0.67	2.09 ± 0.39	1.41 ± 0.24	-
Pseudo specific growth rate						
Mean ± SD	1.40 ± 0.29	1.29 ± 0.20	1.22 ± 0.45	1.38 ± 0.26	0.77 ± 0.24	-
Total length of the whole batch of fish (cm, mean values ± SD): 4.18 ± 0.12						
Body total weight of the whole batch of fish (g, mean values ± SD): 0.78 ± 0.09						

[1] observation on each working day

[2] dead fish are added to the sum of fish with symptoms

[3] one fish died due to attacks of one other fish

SD: Standard deviation

Intoxication symptoms: AK: strongly extended gills, AP: apathy, BA: distended abdomen, DC: dark colouration, FV: fins clearly shortened or frayed out at the border, GA: exopthalmus, KR: convulsions, OB: fish mainly at the water surface, SA: mucous secretion, SR: fish lying on side or back on the bottom, SV: strong ventilation, TS: tumbling during swimming

*: Statistically significant difference from pooled controls (ά = 0.05) when tested with DUNNETT's test.

Table A7_4_3_2-8: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	**Not fulfilled**
Concentration of dissolved oxygen > 60% saturation throughout the test	**X**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**X**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**Not applicable**	
Test substance concentrations maintained within ± 20% of mean measured values		**X ***
No effect on survival nor any other adverse effect found in solvent control	**X**	
Further criteria for poorly soluble test substances	**Not applicable**	

* Results based on measured values which corresponds to 66.28% of the nominal concentrations

Section 7.4.3.3.1 **Annex Point IIIA XIII.2.3** **Section 7.4.3.3.2** **Annex Point IIIA XIII.2.3**	**Bio-accumulation in an appropriate species of fish/ Bio-accumulation in an appropriate invertebrate species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	[redacted]	
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	December 2011	
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*	

Section 7.4.3.3.1 **Annex Point IIIA XIII.2.3** **Section 7.4.3.3.2** **Annex Point IIIA XIII.2.3**	**Bio-accumulation in an appropriate species of fish/ Bio-accumulation in an appropriate invertebrate species**
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species (02)

Annex Point IIIA XIII.2.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Weyers, A. (2007): Preventol CMK Pastillen - *Daphnia magna* Reproduction Test. Bayer Industry Services GmbH & Co. OHG, Leverkusen, Germany, Project-No. 2006/0025/10, date: 2007-03-08 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC Methods for Determination of Ecotoxicity, Annex to Directive 97/548/EEC Part C, Method 20 '*Daphnia magna* Reproduction Test' (2001); (equal to OECD Guideline No. 211 (1998))	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Preventol CMK Pastillen (4-chloro-3-methylphenol)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility: ca. 4 g/L at 20°C	
3.1.6	Method of analysis	HPLC & UV/VIS-detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	-	X
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	
3.4.2	Test organisms	See table A7_4_3_4-3	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species (02)
Annex Point IIIA XIII.2.4

3.4.3	Handling of offspring	Neonates were separated from their parent *Daphnia* by filtration See further details in tables A7_4_3_4-3 and -4.	
3.4.4	Test system	See table A7_4_3_4-4	
3.4.5	Test conditions	See table A7_4_3_4-5	X
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	Survival of parent animals and number of offspring.	
3.4.8	Examination / Sampling	The mortality of adults and the number of neonates was observed three times per week at the renewals of the test media. The pH and oxygen concentration of the controls and the test concentrations were measured at all treatment periods at the beginning and end of the respective periods.	
3.4.9	Monitoring of TS concentration	The concentrations of the test item were determined at test start (day 0) and on day 7 and 14 (new medium) and on day 2, 9, and 16 (aged medium). From the stock solution aliquots were taken at day 0, 2, 5, 7, 9, 12, 14, 16 and day 18.	
3.4.10	Statistics	The results were statistically evaluated according to Welch-T test for inhomogenous variances with Bonferroni adjustement.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentrations	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	The nominal initial concentrations of the test substance were: 0.01, 0.032, 0.1, 0.32 and 1.0 mg/L.	
4.2.2	Actual concentrations of test substance	Mean recoveries of the initial concentrations of CMK were measured on day 0, 7 and 14. The aged test solutions were measured on day 2, 9 and 16. Recovery rates ranged from 91.5 to 100.6% of the nominal values in the freshly prepared media and from 59.5 to 96.3% in the aged media. Measured concentrations below 80% were only found in the lowest concentration of 0.01 mg/L. See table A7_4_3_4-6	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species (02)

Annex Point IIIA XIII.2.4

4.2.3	Effect data	Mortality: No mortality was observed in all test concentrations. Reproduction rate: First brood was recognizable in brood pouch of parent daphnids at day 5 in all concentrations tested. Offsprings were counted from day 7 of the exposure period on. Finally at the end of the study, totally 1070, 1140, 1076, 1073, 1057 and 802 live, young daphnids were reproduced by each 10 adult daphnids exposed to untreated, 0.01, 0.032, 0.1, 0.32 and 1.0 mg/L treated test medium. No significant influence of the test article on the reproduction rate was observed up to a concentration of 0.032 mg/L. At a concentration of 1.0 mg/L, a significant inhibition of the reproduction was observed. Thus, the NOEC and LOEC for reproduction were set at 0.32 mg/L and 1.0 mg/L. No daphnids died after 21 days, thus the NOEC and LOEC for mortality were determined to be ≥ 1.0 mg/L and > 1.0 mg/L, respectively. The number of surviving parent daphnids and inhibition of reproduction rate are summarised in table A7_4_3_4-7 and table A7_4_3_4-8, respectively.	X
4.2.4	Concentration / response curve	No concentration-response curve is included in the original report	
4.2.5	Other effects	Not reported	
4.3	**Results of controls**	There was no mortality in the control. In the control the mean number of newborn water fleas per adult over the whole test period was 107 per replicate.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of CMK on the reproduction and survival rate of daphnids was investigated in a 21 days semi-static test. In the study, nominal concentrations in the range from 0.01 to 1.0 mg/L were tested. The mortality and the number of neonates were compared with corresponding parameters in the controls. The test was performed according to the EEC Methods for Determination of Ecotoxicity, Annex to Directive 97/548/EEC Part C, Method 20 '*Daphnia magna* Reproduction Test' (2001), which equals to the OECD Guideline 211 (1998).	
5.2	**Results and discussion**	Results were based on nominal concentrations since recovery rates in the NOEC and concentrations above were > 80%. The no-effect concentration for reproduction was 0.32 mg/L and for mortality ≥ 1 mg/L. The lowest concentration showing toxicity effects on *Daphnia* reproduction was 1.0 mg/L.	X
5.2.1	$NOEC_{reproduction}$ (21 d)	0.32 mg/L	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species (02)

Annex Point IIIA XIII.2.4

5.2.2	$LOEC_{reproduction}$ (21 d)	1.0 mg/L	
5.2.3	$NOEC_{mortality}$ (21 d)	≥ 1.0 mg/L	
5.2.4	$LOEC_{mortality}$ (21 d)	> 1.0 mg/L	
5.2.5	EC_{50}	Not determined	
5.3	**Conclusion**	CMK did not show any effect on the reproduction of daphnids up to a concentration of 0.32 mg/L (NOEC). The LOEC was found to be 1.0 mg/L. The validity criteria were fulfilled and are summarised in table A7_4_3_4-9.	
5.3.1	Reliability	[redacted]	X
5.3.2	Deficiencies	-	

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 2008

Materials and Methods [redacted]

Results and discussion [redacted]

Conclusion [redacted]

Reliability [redacted]

Acceptability [redacted]

Remarks [redacted]

Date	**COMMENTS FROM ...** *(specify)*
Materials and Methods	*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 A7_4_3_4-1: Preparation of TS Solution for Poorly Soluble or Volatile Test Substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	Mortar, ultrasonic bath, magnetic stirrer with a heating surface, folded filters

Table A7_4_3_4-2: Dilution Water

Criteria	Details
Source	Reconstituted water (“M4 medium”)
Salinity	-
Hardness	14.6 – 15.9 °dH (= 260.61 – 283.82 mg/L $CaCO_3$)
pH	7.8 – 8.0
Ca / Mg ratio	Not stated
Na / K ratio	Not stated
Oxygen content	8.4 – 9.0 mg O_2/L
Conductance	Not given
TOC	Not given
Holding water different from dilution water	No

Table A7_4_3_4-3: Test Organisms

Criteria	Details
Strain / Clone	*Daphnia magna*
Source	Strain of Bundesgesundheitsamt Berlin had been maintained in own laboratory (Bayer Industry Services)
Age	< 24 hours
Breeding method	Constant temperature (20 +/- 1°C); 16:8 hour light-darkness photoperiod (illumination: <1000 lux); M4-medium
Kind of food (breed)	Feeding was performed with green algae *Desmodesmus subspicatus*.
Amount of food (breed)	Not given (‘ad libitum’)
Feeding frequency (breed)	Not given (‘ad libitum’)
Pre-treatment	No
Feeding of animals during test	Day 0-7: 0.1 mg C / *Daphnia* / day Day 8-21: 0.2 mg C / *Daphnia* / day

Table A7_4_3_4-4: Test System

Criteria	Details
Test type	Semi-static conditions with three renewals of test medium per week.
Renewal of test solution	The test medium (treated and untreated) was renewed every monday, wednesday and friday. By that, a total of 9 treatments were performed.
Volume of test vessels	150 mL glass beakers
Volume/animal	100 mL test medium / *Daphnia*
Number of animals/vessel	One animal / test beaker
Number of vessels/concentration	10 vessels (replicates) per concentration/control
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test Conditions

Criteria	Details
Test temperature	20.2 to 20.8 °C
Dissolved oxygen	Fresh medium: 8.4 – 8.9 mg/L; aged medium: 8.4 – 9.0 mg/L
pH	Fresh medium: 7.8 – 8.0 Aged medium: 8.0 – 8.1
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not exceeding 800 lux
Photoperiod	16 hour light : 8 hours darkness

Table A7_4_3_4-6: Analysed Concentrations of CMK in Test Solutions

Nominal conc. [mg/L]	Analysed concentrations of CMK					
	Day 0 (fresh)	% of nominal	Day 2 (aged)	% of nominal	Day 7 (fresh)	% of nominal
Control	<0.0008/ <0.0008	-	<0.0008/ <0.0008	-	<0.0008/ <0.0008	-
0.01	0.0096/ 0.0095	95.5	0.0069/ 0.0070	69.5	0.0097/ 0.0096	96.5
0.1	0.0998/ 0.0988	99.3	0.0894/ 0.0894	89.4	0.0984/ 0.0986	98.5
1.0	1.0066/ 1.0060	100.6	0.9613/ 0.9649	96.3	1.0039/ 1.0032	100.4

Nominal conc. [mg/L]	Analysed concentrations of CMK					
	Day 9 (aged)	% of nominal	Day 14 (fresh)	% of nominal	Day 16 (aged)	% of nominal
Control	<0.0008/ <0.0008	-	<0.0008/ <0.0008	-	<0.0008/ <0.0008	-
0.01	0.0080/ 0.0080	80.0	0.0091/ 0.0092	91.5	0.0058/ 0.0061	59.5
0.1	0.0867/ 0.0871	86.9	0.0990/ 0.0987	98.9	0.0860/ 0.0858	85.9
1.0	0.9117/ 0.9114	91.2	0.9914/ 0.9918	99.2	0.9231/ 0.9240	92.4

Table A7_4_3_4-7: Immobilisation/mortality rate of parent daphnids exposed to CMK (nominal concentrations)

Test item concentration [mg/L]	Immobilisation/mortality rate of parent *Daphnia*	
	absolute	%
Control	0	0
0.01	0	0
0.032	0	0
0.1	0	0
0.32	0	0
1.0	0	0

Table A7_4_3_4-8: Mean number of offsprings per adult reproduced within 21 days

Test item concentration [mg/L]	Mean reproduction rate	Standard deviation	Coefficient of variation (%)
Control	107.0	8.6	8.0
0.01	114.0	8.7	7.6
0.032	107.6	11.0	10.2
0.1	107.3	13.1	12.2
0.32	105.7	7.4	7.0
1.0	80.2	13.2	16.4

Table A7_4_3_4-9: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	Fulfilled	Not fulfilled
Mortality of parent animals in the controls < 20% at test termination	**X**	
Mean number of live offspring produced per parent animal surviving at the end of the test is ≥ 60	**X**	

Section 7.4.3.5.1 **Annex Point IIIA 13.2**	**Effects on sediment dwelling organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	[redacted]	X
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	April 2008	
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		

Section 7.4.3.5.2 **Annex Point IIIA 13.2**	**Aquatic plant toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	[redacted]	
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	April 2008	
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		

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

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1 REFERENCE

1.1	**Reference**	Reis, K.-H. (2007): Effects of 4-Chloro-3-methylphenol (Preventol CMK) on the activity of the soil microflora in the laboratory. Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany. Unpublished Report No. 32322080, date: 2007-04-12.
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test, Guideline 217, January 21, 2000. OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Guideline 216, January 21, 2000.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Carbon transformation test: sampling was done on day 28 and 29 Reason: Sampling at day 28 was done but the data transfer from measuring heads was disturbed, the respiration curves could not be calculated. No presumed effects on the study, because the sampling was repeated immediately (day 29) and the results show clearly that the respiration rates on day 28 could not have been significantly different from day 29.

3 MATERIALS AND METHODS

3.1	**Test material**	4-Chloro-3-methylphenol (Preventol CMK)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	HPLC
3.2	**Reference substance**	Yes Sodium chloride
3.2.1	Method of analysis for reference substance	Not mentioned in the report

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

3.3	**Testing procedure**		
3.3.1	Soil sample / inoculum / test organism	see table A7_5_1_1-1	
3.3.2	Test system	The effect of 1.9 and 19 mg Preventol CMK/kg dry wt soil (corresponding to 1 and 10 times the maximum PEC) on microbial soil respiration and nitrification was compared to an untreated control. Respiration was determined after 0 (within 6 hours), 7, 14 and 29 days, nitrification and ammonification were determined within 6 hours after application and at day 7, 14, 28, 43, 56 and 70. see table A7_5_1_1-2	X
3.3.3	Application of TS	see table A7_5_1_1-3 a) A loamy sand soil was exposed for 29 d to concentrations of 1.9 and 19 mg Preventol CMK/kg dry weight soil (corresponding to maximum PEC and 10 times the maximum PEC). Glucose was added to soil samples to induce maximum respiration rate (2 g/kg natural soil). b) A loamy sand soil was exposed for 70 d to concentrations of 1.9 and 19 mg Preventol CMK/kg dry weight soil (corresponding to maximum PEC and 10 times the maximum PEC). An amount of 0.5% Lucerne meal (related to soil dry weight) was added to the soil to stimulate nitrogen transformation.	X
3.3.4	Test conditions	see table A7_5_1_1-4	
3.3.5	Test parameter	a) effects of O_2-consumption b) effects on NO_3-nitrogen production	
3.3.6	Analytical parameter	a) CO_2 measurement in the respiration test: CO_2 was measured with a BSB-Sensomat System. b) Ammonium and nitrate/nitrite measurement: NH_4-, NO_2- and NO_3-nitrogen were determined by means of a Dionex ion chromatography system (DX-120 IC, AS 50 autosampler, ECD and UVD 340S UV photometer)	
3.3.7	Duration of the test	a) 29 days b) 70 days	
3.3.8	Sampling	Respiration was determined over a period of 6 h after test start, after 7, 14 and 29 days. Nitrification and ammonification were determined after 6 hours after test start, after 7, 14, 28, 43, 56 and 70 days.	
3.3.9	Monitoring of TS concentration	No	
3.3.10	Controls	Deionised water control	
3.3.11	Statistics	Test for normality and homogeneity of variance using the R/S-Test (α = 0.05) and Cochran's-Test (α = 0.05). Where the criterion of homogeneity was fulfilled, the Student-t-test (pair-wise comparison, two sided, α = 0.05) was used for comparison of treated and control values.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

4.1.1	Concentration	Not applicable
4.1.2	Effect data	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1.9 and 19 mg Preventol CMK/kg dry wt soil (corresponding to the maximum PEC and 10 times the maximum PEC)
4.2.2	Actual concentrations of test substance	No measurements were done as it is no requirement of the guideline. All results based on nominal concentrations.
4.2.3	Growth curves	Not applicable
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	Not applicable
4.2.6	Effect data	a) During the 29 day experiments, Preventol CMK applied at 1.9 mg a.s./kg dry weight soil (corresponding to maximum PEC) and a 10 fold overdose had no influence on soil respiration after addition of glucose to a loamy sand soil. b) During the 70 day experiments, Preventol CMK applied at 1.9 mg a.s./kg dry weight soil had no influence on soil nitrogen turnover (given as nitrate content and nitrate formation rate) to a loamy sand soil. The 10 fold overdose had a tolerable impact after 70 days of exposure. During the test, nitrite and ammonium were found only at relatively low concentrations. See tables A7_5_1_1-5a - d for detailed results.
4.2.7	Other observed effects	-
4.3	**Results of controls**	No adverse effects were observed in the controls. See also tables A7_5_1_1-5a - d
4.4	**Test with reference substance**	
4.4.1	Concentrations	Sodium chloride was applied at a rate of 16 g/kg dry soil in a separate study within one year of the start of the experimental phase of this study.
4.4.2	Results	Sodium chloride had a significant impact on nitrogen turnover and respiration activities of soil microflora.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	a) A loamy sand soil was exposed for 29 d to concentrations of 1.9 and 19 mg Preventol CMK/kg dry weight soil (corresponding to the maximum PEC and 10 times the maximum PEC). Glucose was added to soil samples to induce maximum respiration rate (2 g/kg moist soil). 3 replicates per treatment and concentration were compared to a non

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

		treated soil during a 29 d study period. A BSB-Sensomat System was used to determine the CO_2-production over a period of up to 24 hours at different sampling intervals. b) To determine the effect on microbial nitrification Lucerne meal was added to the soil (concentration in soil 0.5%) to stimulate nitrogen transformation. 3 replicates per treatment and concentration were compared to a non treated soil during a 70 d test period. NH_4-, NO_2- and NO_3-nitrogen formed from the nitrification process were determined by means of a Dionex ion chromatography system. Tests were performed according to OECD-Guideline for the Testing of Chemicals, Soil Micoorganisms: Carbon Transformation Test, Guideline 217, January 21, 2000 Nitrogen Transformation Test, Guideline 216, January 21, 2000	
5.2	**Results and discussion**	a) Preventol CMK has no impact on respiration activites of soil microflora when applied up to 19 mg/kg dry weight soil (corresponding to 10 times the maximum PEC). b) Preventol CMK had no impact on soil nitrogen turnover (given as nitrate content and nitrate formation rate) in the test concentration of 1.9 mg/kg dry weight soil (corresponding to maximum PEC) and a tolerable impact in the test concentration of 19 mg/kg dry weight.	
5.2.1	NOEC	≥ 1.9 mg a.i./kg dry weight soil	X
5.2.2	EC_{10}	Not determined	
5.2.3	EC_{50}	≥ 19 mg a.i./kg dry weight soil	
5.3	Conclusion	Preventol CMK does not have long term influence on soil microflora when applied up to 19 mg/kg dry weight soil. The test is considered valid.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

Remarks	[redacted]

	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 A7_5_1_1-1: Properties of the soil sample

Criteria	**Details**
Nature	Loamy sand
Source	District authority: Darmstadt-Dieburg Municipality: 64380 Rossdorf, Germany
Geographical reference on the sampling site	Longitude: 8° 45’ 28’’ E Latitude: 49° 51’ 53’’ N
Data on the history of the site	Soil has not been subjected to any pesticide or fertilizer treatment for at least 2 years.
Use pattern	The field had been left fallow since 2000.
Depth of sampling [cm]	5 - 20
Sand / Silt / Clay content [% dry weight]	Classification DIN 19683 Clay (%) < 2 µm 9.0 Silt (%) 63 µm to ≥ 2µm 31.7 Sand (%) ≥ 63 – 2000 µm 59.3
pH	6.9
Organic carbon content [% dry weight]	1.1
Nitrogen content [~~%~~ **mg/kg** dry weight]	~~0.001986~~ **19.86**
Cation exchange capacity [mval/100 g dry wt soil]	44 mmol Ba/kg dry weight
Initial microbial biomass [mg C/kg dry wt soil]	910
Reference of methods	Not mentioned in the report
Collection / storage of samples	Soil samples were collected from fallow grassland, were air dried and sieved through a 2 mm sieve at room temperature. The samples were stored at 20 ± 2 °C for 1 week and then stored cooled in a refrigerator **during 2 weeks**.
Preparation of inoculum for exposure	n. a.
Pretreatment	n. a.

Table A7_5_1_1-2: Test system for soil respiration / nitrification tests

Criteria	Details
Culturing apparatus	a) Carbon transformation: After mixing, soil was incubated in 1 L disposable plastic boxes b) Nitrogen transformation: After mixing, soil was incubated in 0.5 L disposable plastic boxes
Number of vessels / concentration	3 units per treatment group
Aeration device	Plastic boxes were covered by perforated lids
Measuring equipment	a) Pressure decrease in the reaction vessels was measured up to 24 consecutive hours using the BSB Sensomat system, Aqualytic Langen. The oxygen consumption and the carbon dioxide release were calculated there from. b) The nitrogen content was determined using a Dionex DX 120 ion chromatograph and associated equipment.
Test performed in closed vessels	Yes

Table A7_5_1_1-3: Application of test substance and sampling

Criteria	Details
Application procedure	An aqueous suspension in deionised water was prepared and mixed into the soil by means of a laboratory mixer.
Carrier	Not applicable
Concentration of liquid carrier [% v/v]	Not applicable
Liquid carrier control	Water
Sampling procedure	a) Respiration was determined after 0 (within 6 hours), 7, 14 and after 29 days. Therefore, 100 g soil were sampled, mixed with 2 g/kg (moist soil) of glucose (0.8 mL of a solution of 250 g glucose/L deionised water). The amount of glucose was determined to give the highest respiration rates (determination at a soil batch of the test soil). The glucose amended soil samples were incubated at 20 ± 2 °C. the pressure decrease in the reaction vessels was measured up to 24 consecutive hours using the BSB Sensomat system, Aqualytic Langen. The oxygen consumption and the carbon dioxide release were calculated there from. b) For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (7, 14, 28, 43, 56 and 70 days). The nitrogen content was determined in each sample of treated and control soils using a Dionex DX 120 ion chromatograph.

Table A7_5_1_1-4: Test conditions

Criteria	Details
Organic (inorganic) substrate	Addition of: a) 2 g/kg (moist soil) of glucose (0.8 mL of a solution of 250 g glucose/L deionised water) b) 0.5% Lucerne meal (related to soil dry weight)
Incubation temperature	~~20 ± 22 °C~~ **20 ± 2°C**
Soil moisture	49 – 52% of its maximum water holding capacity
Method of soil incubation	Bulk
Aeration	-

TableA7_5_1_1-5a: Effects of Preventol CMK on soil respiration (test a) in a loamy sand soil (sampling after 29 days)

Day	Control			Preventol CMK [mg a.i./kg dry wt soil]					
				1.9			19		
	Soil respiration [1]	SD	CV	Soil respiration [1]	SD	% [2]	Soil respiration [1]	SD	% [2]
0	12.721	0.340	2.67	12.073	0.329	-5.09 (n.s.)	12.322	0.418	-3.14 (n.s.)
7	10.810	1.203	11.13	11.683	0.177	8.08 (n.s.)	10.923	1.108	1.05 (n.s.)
14	11.892	0.332	2.79	11.516	1.009	-3.16 (n.s.)	11.756	0.127	-1.14 (n.s.)
29	11.397	0.348	3.05	11.459	0.085	0.54 (n.s.)	10.662	0.568	-6.45 (n.s.)

[1] soil respiration [mg carbon dioxide / (hour * kg dry wt soil)], average from three samples

[2] % deviation of corresponding control

SD: Standard deviation

CV: Coefficient of variation = SD / mean value * 100

n.s.: not statistically significant (according to Student-t-test, two sided $\alpha = 0.05$)

TableA7_5_1_1-5b: Effects of Preventol CMK on soil ~~nitrogen transformation~~ nitrate content (test b) in a loamy sand soil (test duration 70 days)

Sampling	Control		Preventol CMK [mg a.i./kg dry wt soil]			
			1.9		19	
	NO_3 – Nitrogen [mg / kg dry wt soil] mean values					
	Nitrate-N content	Replicate Variation CV [1]	Nitrate-N content	% Deviation [2]	Nitrate-N content	% Deviation [2]
Day 0	16.950	1.11	16.234 *	-4.22	16.797	-0.90
Day 7	8.055	13.78	4.936 *	-38.72	1.335 *	-83.43
Day 14	11.925	2.60	8.041 *	-32.57	4.445 *	-62.73
Day 28	25.757	0.73	22.558 *	-12.42	18.145 *	-29.55
Day 42	47.081	3.37	44.529 *	-5.42	38.026 *	-19.23
Day 56	59.574	3.31	55.868	-6.22	46.391 *	-22.13
Day 70	74.554	2.47	70.305	-5.70	64.118 *	-14.00

[1] Replicate variation CV = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value * 100)
[2] % deviation to control
+ stimulating effect
- inhibitory effect
* statistically significant different from control (Student-t-test, α = 0.05)

TableA7_5_1_1-5c: Effects of Preventol CMK on soil ~~respiration~~ nitrate transformation (test b) in a loamy sand soil (test duration 70 days)

Interval [3]	Control		Preventol CMK [mg a.i./kg dry wt soil]			
			1.9		19	
	NO_3 – Nitrogen Formation Rate [mg / kg dry wt soil per day] [3]					
	Nitrate-N formation	Replicate Variation CV [1]	Nitrate-N formation	% Deviation [2]	Nitrate-N formation	% Deviation [2]
Day 0 – 7	-1.27	-10.94	-1.61 *	26.8	-2.21 *	74.0
Day 0 – 14	-0.36	-10.00	-0.58 *	61.1	-0.88 *	144.4
Day 0 – 28	0.32	1.88	0.23 *	-28.1	0.05 *	-84.4
Day 0 – 42	0.72	4.44	0.67	-6.9	0.50 *	-30.6
Day 0 – 56	0.76	4.21	0.71	-6.6	0.53 *	-30.3
Day 0 - 70	0.83	2.77	0.77	-7.2	0.67 *	-19.3

[1] Replicate variation CV = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value * 100)
[2] % deviation to control
[3] cumulative calculation
+ stimulating effect
- inhibitory effect
* statistically significant different from control (Student-t-test, α = 0.05)

TableA7_5_1_1-5d: Effects of Preventol CMK on soil mineral nitrogen content ~~transformation~~ (test b) in a loamy sand soil (test duration 70 days)

Sampling	Control		Preventol CMK [mg a.i./kg dry wt soil]			
			1.9		19	
	Mineral Nitrogen [mg / kg dry wt soil ~~per day~~] mean values [3]					
	Mineral-N content	Replicate Variation CV [1]	Mineral-N content	% Deviation [2]	Mineral-N content	% Deviation [2]
Day 0	24.780	1.11	23.969 *	-3.27	23.673 *	-4.47
Day 7	9.616	12.19	6.481 *	-32.60	2.822 *	-70.65
Day 14	12.924	2.82	9.219 *	-28.67	5.585 *	-56.79
Day 28	26.516	0.71	23.321 *	-12.05	18.878 *	-28.81
Day 42	49.973	3.51	47.603	-4.74	40.928 *	-18.10
Day 56	60.330	3.13	56.668	-6.07	47.071 *	-21.98
Day 70	75.804	2.46	71.501	-5.68	65.197 *	-13.99

[1] Replicate variation CV = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value * 100)
[2] % deviation to control
[3] sum of ammonium-N, nitrite-N and nitrate-N
+ stimulating effect
- inhibitory effect
* statistically significant different from control (Student-t-test, $\alpha = 0.05$)

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

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		1 REFERENCE
1.1	**Reference**	Schulz, L. (2012): Preventol CMK – Effects on the activity of soil microflora (Nitrogen transformation test). BioChem agrar, Labor für biologische und chemische Analytik GmbH 04827 Gerichshain, Germany, Project-No. 12 10 48 011 N, date: 2012-04-13 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes; OECD Guideline 216 (2000)
2.2	**GLP**	No (all raw data attached to the report)
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Preventol CMK (p-chloro-m-cresol)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	n.a
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	Gas chromatography
3.2	**Reference substance**	Dinoterb (in a separate study, see section 4.4).
3.2.1	Method of analysis for reference substance	-
3.3	**Testing procedure**	
3.3.1	Soil sample / inoculum / test organism	See table A7_5_1_1-1.
3.3.2	Test system	Experimental phase: 15.12.2011 to 26.03.2012. The effect of the test substance at concentrations 1, 5, 10, 30, 60, 100, 300, and 1000 mg a.s./kg dry weight (d.wt.) soil on nitrogen transformation was compared to an untreated control. Each test concentration was tested with three replicates each. The loamy sand soil was collected from fallow ground and sieved through a 2 mm sieve.

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

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		200 g dw soil per test vessel was weighed. The soil was mixed with 0.5% (i.e. 1.0 g/200 g dw soil) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 15.6/1). Since the water solubility was low, the test item was thoroughly mixed with quartz meal. Subsequently the obtained mixture was added and mixed with the soil by means of a hand stirrer. For an optimum distribution of the test item in the soil, the test item quartz meal mixture was applied at a ratio of about 10 g/kg dw soil. The incubation of the prepared soil was carried out in wide mouth glass flasks (500 mL) under the conditions mentioned in table A7_5_1_1-4 for 100 days. The screw caps of the flask permitted an air exchange. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40-50% of WHC with deionised water. Control samples were not treated with the test item, i.e. were prepared with quartz meal only. For the reference test, samples were treated with the reference item dinoterb in a separate test. Nitrogen mineralization and nitrification was determined after 0 (within 3 hours), 14, 28 and 100 days. See also table A7_5_1_1-2.
3.3.3	Application of TS	Since the water solubility was low, the test item was thoroughly mixed with quartz meal. Subsequently the obtained mixture was added and mixed with the soil by means of a hand stirrer. See also table A7_5_1_1-3.
3.3.4	Test conditions	See table A7_5_1_1-4.
3.3.5	Test parameter	The microbial conversion of organic nitrogen to nitrate is a multi-step process. In a primary reaction, soil organic matter is mineralised to ammonia (ammonification, mineralization). Furthermore, ammonia is converted via nitrite to nitrate, a process which is designated as nitrification. This step represents the second step in organic matter conversion. The nitrification processes are considered to be important for soil fertility. In the study the soil nitrate content and the nitrate formation rate was determined.
3.3.6	Analytical parameter	Ammonium, nitrite and nitrate measurement.
3.3.7	Duration of the test	100 days
3.3.8	Sampling	Level of nitrification was determined for all dose levels at intervals of 0 (within 3 hours), 14, 28 and 100 days after treatment. The concentration of ammonium, nitrite and nitrate was determined for each sampling interval in a 1 M KCl extract of the soil sample.
3.3.9	Monitoring of TS concentration	No
3.3.10	Controls	Untreated control
3.3.11	Statistics	Statistical evaluation of the test results was performed with Dunett-t-test, one-sided smaller ($\alpha = 0.05$) and Probit analysis using linear weight

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		regression for day 28 and day 100 after application. Statistical programme: ToxRat Professional 2.10 (2009).
		4 RESULTS
4.1	**Range finding test**	not performed
4.1.1	Concentration	n.a.
4.1.2	Effect data	n.a.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1, 5, 10, 30, 60, 100, 300, and 1000 mg test item per kg d.wt. soil.
4.2.2	Actual concentrations of test substance	No measurements were done.
4.2.3	Growth curves	-
4.2.4	Cell concentration data	-
4.2.5	Concentration/ response curve	Presented in the raw data of the report.
4.2.6	Effect data	The test item caused a maximum inhibition of -60.7% and -72.2% at 1000 mg/kg dw soil 28 days and 100 days after application, respectively. The NOEC of the test item was calculated to be 300 mg/kg dw soil 28 days and 100 days after application. The EC_{50} was calculated to be 555.1 mg/kg and 395.1 mg/kg dw soil 28 days and 100 days after application, respectively. See table A7_5_1_1-5 for detailed results.
4.2.7	Other observed effects	-
4.3	**Results of controls**	See table A7_5_1_1-5
4.4	**Test with reference substance**	Yes, with “dinoterb” (purity: [redacted]). It was tested in a separate study to verify the sensitivity of the test system.
4.4.1	Concentrations	6.80, 16.00 and 27.00 mg/kg.
4.4.2	Results	The reference substance showed a clear influence on the microflora (stimulation), thereby showing the sensitivity of the test. See results in table A7_5_1_1-6.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The purpose of this study was to determine a dose-response relationship (EC_{50}) for the activity of soil microflora with regard to nitrogen transformation (mineralization) in a laboratory test over a period of 100 days of exposure. The test was performed in accordance with OECD test

X

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

		guideline 216 (2000) by measuring the nitrogen turnover. The influence of Preventol CMK on soil microorganisms was determined by measuring the microbial nitrification in one soil (batch 4/2011; Wassergut Canitz, Germany). In the study, the loamy sand soil enriched with lucerne meal (concentration in soil 0.5%) was moistened to 45% of its maximum water-holding capacity and incubated in the dark at 20 ± 2°C following treatment with the test item. Control soil was not treated with the test item, but was incubated in parallel under identical conditions as for the treated soil. The test consisted of 10 treatment groups (including control) with three replicates each. Test item concentrations were the following: 1, 5, 10, 30, 60, 100, 300, and 1000 mg test item per kg d.wt. soil. Nitrogen transformation (ammonium, nitrate and nitrite) was determined on day 0 (after approx. 3 hours) and at intervals of 14, 28 and 100 days after application.	
5.2	**Results and discussion**	Since differences in nitrate content between treated and untreated soil were greater than the OECD trigger value of 25, measurements were continued to a maximum of 100 days.	X
5.2.1	NOEC	300 mg/kg dw soil	X
5.2.2	EC_{10}	Not determined	
5.2.3	EC_{50}	555.1 mg/kg dw soil (after 28 d); 395.1 mg/kg dw soil (after 100 d).	
5.3	Conclusion	The test item caused a maximum inhibition of -60.7% and -72.2% at 1000 mg/kg dw soil 28 days and 100 days after application, respectively. The coefficients of variation in the control were maximum 1.9% and thus fulfilled the demanded range (≤ 15%). The results on the reference substance in the most recent test demonstrate the sensitivity of the test system.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	-	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 June 2012
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_5_1_1-1: Properties of the soil sample X

Criteria	Details
Nature	Biologically active agricultural soil (loamy sand according to DIN).
Source	Wassergut Canitz, Germany, batch 4/2011
Geographical reference on the sampling site	The soil was freshly sampled on October 05, 2011 (latitude 51.403774567° N, longitude 12.694435960° E) in Wassergut Canitz, Germany.
Data on the history of the site	Fallow ground (2010-2011)
Use pattern	Last application of plant protection products: 1990. No application of fertilisers since 2003.
Depth of sampling [cm]	The soil was removed to a depth of 20 cm.
Sand / Silt / Clay content [% dry weight]	Classification DIN 4220/ISO 11277: loamy sand < 2 µm (clay) 8.1 63 to ≥ 2 µm (silt) 38.9 ≥ 63 µm to 2000 µm (sand) 53.0 Classification USDA: sandy loam < 2 µm (clay) 8.1 50 to ≥ 2 µm (silt) 37.3 ≥ 63 µm to 2000 µm (sand) 54.6
pH	6.6 Measured pH values during the test: 6.1-6.8
Organic carbon content [% dry weight]	1.47
NH_4^+-N (mg/kg dry weight) NO_2^--N (mg/kg dry weight) NO_3^--N (mg/kg dry weight) Total N (mg/kg dry weight)	n.a. n.a. n.a. 0.14
Cation exchange capacity [mmol Ba/kg dry wt soil]	86
Initial microbial biomass [mg C/kg dry wt soil]	27.26 (1.85% of total organic carbon)
Reference of methods	OECD Guideline 216 (2000)
Collection / storage of samples	Mixed sample was **dried at room temperature for 5 days and** passed through a 2 mm mesh sieve. **Samples were stored at 4°C for 7-8 weeks** and then adapted to test conditions.
Preparation of inoculum for exposure	01.12 – 15.12.2011 conditioning under test conditions. Then soil was filled into incubation flasks and equilibrated at 20 ± 2°C in the dark. The soil moisture content was adjusted to 45 of the maximum water holding capacity (MWC) prior to application.
Pretreatment	n.a.

Table A7_5_1_1-2: Test system:

Criteria	Details
Culturing apparatus	Wide mouth glass flasks
Number of vessels / concentration	3
Aeration device	The screw caps of the flask permitted an air exchange.
Measuring equipment	The concentrations of ammonium, nitrite and nitrate were measured using an Autoanalizer (produced by BRAN+LUEBBE, Hamburg, Germany).
Test performed in closed vessels	Yes (soil covered by screw caps)

Table A7_5_1_1-3: Application of test substance and sampling:

Criteria	Details
Application procedure	The test item was thoroughly mixed with quartz meal. Subsequently the obtained mixture was added and mixed with the soil by means of a hand stirrer.
Carrier	n.a.
Concentration of liquid carrier [% v/v]	n.a.
Liquid carrier control	n.a.
Sampling procedure	Nitrification was determined at the beginning (day 0), 14, 28 and 100 days after treatment.

Table A7_5_1_1-4: Test conditions:

Criteria	Details
Organic (inorganic) substrate	Soil samples were amended with 0.5% lucerne meal (related to soil dry weight) before application in the transformation test.
Incubation temperature	20 ± 2 °C in a climatic room. Measured values during the test: 18.9 to 21.6°C
Soil moisture	Approx. 45% of the maximum soil water holding capacity. Measured values during the test: 15.76 – 17.27 g/100 g dry soil.
Method of soil incubation	Series of individual and equally sized subsamples of each treatment group in darkness.
Aeration	-

Table A7_5_1_1-5: Effects on nitrogen transformation (nitrate content) in soil amended with lucerne meal after treatment with the test item:

Treatment group (mg test item/kg soil dry weight)		Days after application			
		0	14	28	100
Control	NO_3-N [mg/100 g soil d.w.]	1.96	3.33	4.19	6.67
1	NO_3-N [mg/100 g soil d.w.]	1.91	3.20	4.14	6.76
	Deviation from control [%][1]	-2.9	-3.7	-1.4	+1.3
5	NO_3-N [mg/100 g soil d.w.]	1.88	3.02	4.03	7.49
	Deviation from control [%][1]	-4.2	-9.3	-3.9	+12.3
10	NO_3-N [mg/100 g soil d.w.]	1.78	3.16	4.09	7.34
	Deviation from control [%][1]	-9.5	-5.0	-2.5	+10.1
30	NO_3-N [mg/100 g soil d.w.]	1.82	3.25	4.24	7.43
	Deviation from control [%][1]	-7.1	-2.3	+1.1	+11.3
60	NO_3-N [mg/100 g soil d.w.]	1.79	3.62	4.74	7.76
	Deviation from control [%][1]	-8.7	+8.9	+13.0	+16.3
100	NO_3-N [mg/100 g soil d.w.]	1.79	3.73	4.78	8.07
	Deviation from control [%][1]	-9.0	+12.1	+14.0	+21.0
300	NO_3-N [mg/100 g soil d.w.]	1.78	1.76	3.91	8.57
	Deviation from control [%][1]	-9.3	-47.2	-6.8	+28.5
600	NO_3-N [mg/100 g soil d.w.]	1.80	1.81	1.85 *	2.19 *
	Deviation from control [%][1]	-8.1	-45.5	-55.8	-67.1
1000	NO_3-N [mg/100 g soil d.w.]	1.79	1.60	1.65 *	1.86 *
	Deviation from control [%][1]	-9.0	-52.0	-60.7	-72.2

The calculations were performed with non-rounded values

1) based on NO_3-nitrogen production; - = inhibition; + = stimulation

* statistically significant differences compared to control (Dunnett-t-test one-sided smaller; $p \leq 0.05$) on day 28, 100

Table A7_5_1_1-6: Influence of the reference item dinoterb on nitrogen transformation:

Sampling date [DAA]	Control	Dinoterb 6.80 mg/kg soil d.w.		Dinoterb 16.00 mg/kg soil d.w.		Dinoterb 27.00 mg/kg soil d.w.	
	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1)]
0	14.5	14.8	+1.6	14.6	+0.2	14.7	+1.4
7	25.6	34.9	+36.2	34.4	+34.4	26.5	+3.6
14	31.1	42.8	+37.9	50.9	+63.8	45.1	+45.2
28	38.1	54.1	+42.0	64.0	+68.1	73.2	+92.3

[1)] based on NO_3-nitrogen production; - = % inhibition; + = % stimulation

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Eisenia fetida

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		1 REFERENCE
1.1	**Reference**	Lührs, U. (2007): Acute Toxicity (14 Days) of 4-Chloro-3-methylphenol (Preventol CMK) to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany, Project No. 32326021, date: 2007-01-17 (unpublished). Lührs, U. (2007): Amendment to Project No. 32326021, date: 2007-03-15 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD-Guideline for the testing of chemicals No. 207 “Earthworm, Acute Toxicity Test“ (adopted April 4, 1984), ISO-Guideline 11268-1:1993 “Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*) - Part 1: Determination of acute toxicity using artificial soil substrate“.
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 METHOD
3.1	**Test material**	4-Chloro-3-methylphenol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	No
3.2	**Reference substance**	With 2-Chloroacetamide
3.2.1	Method of analysis for reference substance	No

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Eisenia fetida

3.3	**Testing procedure**	
3.3.1	Preparation of the test substance	A stock solution was prepared by dissolving 3900 mg of 4-Chloro-3-methylphenol (Preventol CMK) in 20 mL acetone. A sequential 1:2 dilution series was prepared, starting with the 195.0 mg/mL stock solution by adding 7 mL of acetone to 7 mL of the stock solution or dilution. Then 5 mL of the stock solution or corresponding dilution were added to 20 g fine quartz sand. The treated sand was left for at least one hour in a fume hood until the solvent had evaporated and was then mixed with a spoon. The artificial soil was then added to test item blended sand. There were no significant deviations from the target concentration (< 5%). To the control the same amount of acetone and sand was added. While mixing the artificial soil in a laboratory mixer for approximately 5 min the soil of each treatment group was ventilated and moistened with deionised water to achieve the required water content. Each group was treated in one batch and then split into 4 replicates.
3.3.2	Application of the test substance	See above. 500 g dry weight substrate was prepared for each test container.
3.3.3	Test organisms	*Eisenia fetida*, see Table A7_5_1_2-2
3.3.4	Test system	See Table A7_5_1_2-3
3.3.5	Test conditions	See Table A7_5_1_2-4
3.3.6	Test duration	14 days
3.3.7	Test parameter	Effect on mortality of earthworms after exposure over 14 days, behavioural effects, weight change.
3.3.8	Examination	Number of dead earthworms was assessed at days 7 and 14 after application. On day 7 and 14 after application the artificial soil was emptied from the jars onto a tray and the number of live and dead earthworms in each replicate was assessed. Missing earthworms and earthworms failing to respond to gentle stimulation were also considered dead. Live earthworms and soil were placed back into the test containers after the assessment on day 7. Behavioural abnormalities, number of affected earthworms (e.g. lack of movement, rigidity, etc.) were assessed at days 7 and 14 after application. Mean Body Weights per test container were determined at study initiation (day 0) and 14 days after exposure, using the same washing and weighing procedures as at test initiation.
3.3.9	Monitoring of test substance concentration	No
3.3.10	Statistics	Fisher extract test (mortality), Dunnett test (weight change), Probit Analysis (determination of LC_{50}) The software used to perform the statistical analysis was ToxRatPro, version 2.09(2005).

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Eisenia fetida

		4 RESULTS
4.1	**Filter paper test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	On the basis of the results of the non-GLP range finding test, the following concentrations were selected: 15, 30, 60, 60 120 240, 480 mg test item/kg soil dry weight. Control: untreated (same amount of acetone treated quartz sand as in the test item treated groups was added and moistened with deionised water)
4.2.2	Effect data (Mortality and body weight)	see Table A7_5_1_2-5 and A7_5_1_2-6
4.2.3	Concentration / effect curve	It is given in the report on page 19
4.2.4	Other effects	Worms were fidgety wriggling about at the concentration of 120 mg/kg soil after 7 days of exposure. This effect was not observed after 14 days of exposure. No additional behavioural effects were observed in any other concentration.
4.3	**Results of controls**	
4.3.1	Mortality	0 % mortality was observed in the control. See also Table A7_5_1_2-5
4.3.2	Number/ percentage of earthworms showing adverse effects	-
4.3.3	Nature of adverse effects	Abnormalities, e.g. changes in behaviour, were not observed.
4.4	**Test with reference substance**	2-Chloroacetamide
4.4.1	Concentrations	The LC_{50} of 2-Chloroacetamide is determined at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly over time. The most recent test was performed in August 2006 (IBACON Study 26882021).
4.4.2	Results	The LC_{50} after 14 days was determined to be 27.0 mg test item/kg soil dry weight (95% confidence limits of 22.6 and 31.1). This value is within the range recommended by the guideline ISO11268-1, 1993 (20

Section A7.5.1.2 Earthworm, acute toxicity test

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Eisenia fetida

to 80 mg 2-chloroacetamide/kg soil).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test was conducted over 14 days in treated artificial soil. Different concentrations (control, 15, 30, 60, 120, 240 and 480 mg/kg dw soil) of the test item were mixed homogeneously into the soil which was filled in glass vessels before the earthworms were introduced on top of the soil; 6 concentrations and one control; 4 replicates per concentration with 10 worms each. The assessment of worm mortality and behavioural effects were after 7 and l4 days, measurement of weight change as sublethal parameter after 14 days.
5.2	**Results and discussion**	In the 14-days toxicity study with 4-chloro-3-methylphenol (Preventol CMK) to earthworms (*Eisenia fetida*) the LC_{50} was determined to be 139.4 mg test item/kg soil. The NOEC related to mortality and biomass was determined to be 60.0 mg 4-Chloro-3-methylphenol (Preventol CMK)/kg soil dry weight. The study is considered to represent worst case laboratory conditions. The results are nominal concentrations, since an analytical check of the test concentrations for this 14 day acute test is not specified in the guideline.
5.2.1	LC_0	NOEC = 60.0 mg a.i./kg dry weight soil
5.2.2	LC_{50}	139.4 mg a.i./ kg dry weight soil
5.3	**Conclusion**	The mortality rate in the control was below 10% which is regarded as the limit for natural mortality (see validity criteria in Table A7_5_1_2-7). The loss of biomass in the control did not exceed 20%. Furthermore, the LC_{50} of the reference substance was within the usual range. Thus, the test is considered valid.
5.3.1	Other Conclusions	-
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
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 A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Deionised water
Alkalinity / Salinity	-
Hardness	-
pH	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	No

Table A7_5_1_2-2: Test organisms

Criteria	Details
Species/strain	*Eisenia fetida* (Savigny 1826)
Source of the initial stock	Not specified
Culturing techniques	Bred by IBACON in a breeding medium of cattle manure, peat, sand and straw, mainly fed with cattle manure, stored at room temperature.
Age/weight	Clitellated adult earthworms, less than one year old (weight range 300 to 600 mg, 11 to 12 months) from cultures held at the laboratory.
Food	The animals were not fed during the test
Pre-treatment	1 day, in artificial soil, under test conditions (20°C)

Table A7_5_1_2-3: Test system

Criteria	Details
Artificial soil test substrate	Artificial soil according to OECD 207 but with reduced content of peat (5%). Composition: - 5.0% Sphagnum-peat, air-dried and finely ground (2 mm), (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany) - 20% Kaolin clay (Erbslöh, 65558 Lohrheim, Germany) - approximately 0.2% chalk ($CaCO_3$) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany) - approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwerke Frechen, Postfach 1780, 50207 Frechen, Germany) According to OECD 222 and EPPO 2002, 5% of peat was used in the artificial soil considering the influence of the properties of the test item (LogKow >2) on bioavailability.
Test mixture	-
Size, volume and material of test container	Normal glass bottling jars (1 L), loosely covered by glass-lids to enable exchange of air and to minimise evaporation, filled with approximately 500 g (dry weight equivalent) artificial soil.
Amount of artificial soil (kg)/ container	500 g dry weight
Nominal levels of test concentrations	Control and 15, 30, 60, 120, 240 and 480 mg/kg dw soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Continuous light (410 - 750 Lux)
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	19°C - 22°C
Moisture content	Maximum water holding capacity of the artificial soil: 40% of the dry weight. - Water content of soil dry weight at test initiation 53.3% to 57.5% of the max. water holding capacity. - At test termination 51.8% to 55.3% of the max. water holding capacity.
pH	Initial pH: 5.6-5.7 End of the study: 5.3-5.5
Adjustment of pH	Yes; approximately 0.2% chalk ($CaCO_3$) was added to adjust pH to 6.0 ± 0.5
Light intensity / photoperiod	410-750 Lux, continuous light
Relevant degradation products	Degradation products were not investigated in this study

Table A7_5_1_2-5: Results obtained in the study with p-chloro-m-cresol after 7 and 14 days

Concentration [mg a.i./kg d. wt.s.]	Mortality 7 days [%]			Mortality 14 days [%]		
	Mean	SD	significance	Mean	SD	significance
Control	0.0	0.0	-	0.0	0.0	-
15.0	0.0	0.0	-	0.0	0.0	-
30.0	0.0	0.0	-	0.0	0.0	-
60.0	0.0	0.0	-	0.0	0.0	-
120	5.0	10.0	n.s.	17.5	35.0	*
240	100.0	0.0	*	100.0	0.0	*
480	100.0	0.0	*	100.0	0.0	*

- not relevant
n.s. not significantly different compared to the control, Fischer exact test, $\alpha = 0.05$
*significantly different compared to the control, Fischer exact test, $\alpha = 0.05$

Table A7_5_1_2-5: Earthworm body weight changes

Concentration [mg a.i./kg d. wt.s.]	Test start	After 14 days		
	Mean [mg/worm]	Mean [mg/worm]	Difference %[1)]	Significance
Control	404.3	370.8	-7.7	-
15.0	397.1	353.5	-10.7	n.s.
30.0	397.5	362.3	-8.7	n.s.
60.0	404.2	375.5	-7.1	n.s.
120	407.1	279.9	-31.2	*
240	411.1	/	-	-
480	417.6	/	-	-

1) % mean of 4 replicates
n.s. not significantly different compared to the control, Dunnett test, $\alpha = 0.05$
*significantly different compared to the control, Dunnett test, $\alpha = 0.05$

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD Guideline 207

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	**X**	

Section 7.5.1.3 Terrestrial plant toxicity
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Official use only

		1 REFERENCE
1.1	**Reference**	Buetzler, R., Meinerling, M. (2007): Effects of Preventol CMK on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test. IBACON GmbH, Rossdorf, Germany, Report No. 32327086, Date: 2007-02-05.
1.2	**Data protection**	Yes
1.2.1	Data owner	LANXESS Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes; OECD Guideline 208 (Proposal for Updating Guideline 208, Draft Document March 2005): Seedling Emergence and Seedling Growth Test
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	Preventol CMK
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	Soluble in acetone (according to Ibacon personal)
3.1.6	Method of analysis	HPLC
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since test item is soluble in acetone
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	
3.4.1	Dilution water	See Table A7_5_1_3-2
3.4.2	Test plants	See Table A7_5_1_3-3

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3.4.3	Test system	See Table A7_5_1_3-4; After application of the test substance into the soil, pots were filled with the soil and seeds were sown into the contaminated soil.
3.4.4	Test conditions	See Table A7_5_1_3-5
3.4.5	Test duration	Exposure time: 14 days after application
3.4.6	Test parameter	Fresh weight; Germination; Mortality; Phytotoxicity (e.g. chlorosis, necrosis, abnormal growth); Growth stages; See Tables A7_5_1_3-4 and A7_5_1_3-5 for details.
3.4.7	Sampling	Fresh weight: 14 days after 50 % seedling emergence in the control, Germination: daily until 50 % of the control plants had emerged; further checks were done weekly, Mortality: 14 days after 50 % seedling emergence in the control, Phytotoxicity: weekly after 50 % seedling emergence in the control; Growth stages (BBCH): Day 14; See also Tables A7_5_1_3-4 and A7_5_1_3-5 for details.
3.4.8	Method of analysis of the plant material	Not applicable
3.4.9	Quality control	Yes (Test was performed according to GLP by certified laboratory)
3.4.10	Statistics	<u>Statistical analysis of the control plants and solvent control plants</u>: Fresh weight data of the control and solvent control plants were tested for normal distribution and homogeneity of variance using the Kolmogoroff-Smirnov-Test and the Cochran- Test. Because the data were normally distributed and homogeneous the Student-t-test was used. For the mortality and germination data Fisher's Exact-Test was used. Because there was no statistical difference between the data of the control plants and solvent control plants the results of the plants treated with the test concentrations were compared with the results of the solvent control plants. <u>Statistical analysis of the plants treated with the tested concentrations and the solvent control plants:</u> Fresh weight data were tested for normal distribution and homogeneity of variance using the Kolmogoroff-Smirnov-Test and the Cochran-Test. If the data were normally distributed and homogeneous the Dunnett Test was used for comparing treatment groups and control. If the data were not homogeneous the Bonferroni-Welch-t-test was used. In order to determine the EC_{25} and EC_{50} values, a regression analysis was performed (Probit analysis). For the mortality and germination data Fisher's Exact Test was used. The computer programs used to perform the statistical analyses were ToxRat® SPiRiT Solutions (2005), Version 2.09. The significance level for all tests was $\alpha = 0.05$ (two-sided).

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Applied initial concentration	Based on the results of the non-GLP range finder the following concentrations of Preventol CMK were prepared: *Brassica napus*: mg test item/kg soil dry weight (nominal) 0.96 2.40 6.00 15.0 37.5 93.8 234.4 *Glycine max*: mg test item/kg soil dry weight (nominal) 2.40 6.00 15.0 37.5 93.8 234.4 *Avena sativa*: mg test item/kg soil dry weight (nominal) 15.0 37.5 93.8 234.4	X
4.1.2	Phytotoxicity rating	See Table A7_5_1_3-6b *Brassica napus*: Phytotoxic effect was growth reduction at 37.5 mg Preventol CMK/kg soil dry weight and 93.8 mg/kg. Additionally chlorosis and necrosis were observed at 93.8 mg Preventol CMK/kg. There was a slight chlorosis in one pot each at 2.40 mg/kg and 0.96 mg/kg *Glycine max*: Phytotoxic effect were chlorosis and growth reduction at 93.8 mg Preventol CMK/kg soil dry weight. At 234.4 mg/kg a strong growth reduction was observed. Because one seedling was dead in one pot at 2.40 mg/kg, there was an overall phytotoxic effect of 3.3 % for this concentration. *Avena sativa*: Phytotoxic effects were chlorosis, necrosis and growth reduction.	X
4.1.3	Plant height	Not reported	
4.1.4	Plant dry weights	For fresh weights see Table A7_5_1_3-6a. The most sensitive species in fresh weight was *Brassica napus* (EC_{50}:	

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		27.7 mg a.i./kg soil) followed by *Avena sativa* and *Glycine max* with EC_{50} values of 30.3 and 62.4 mg a.i./kg soil.
4.1.5	Root dry weights	Not described
4.1.6	Root length	Not described
4.1.7	Number of dead plants	See Table A7_5_1_3-6a Most sensitive species for the parameter mortality was *Brassica napus* which showed mortality at 93.8 mg a.i./kg soil.
4.1.8	Effect data	See Table A7_5_1_3-7a (effect data based on results of the fresh weight).
4.1.9	Concentration / response curve	No plot of concentration/response curve given in report.
4.1.10	Other effects	None
4.2	**Results of controls**	
4.2.1	Number/ percentage of plants showing adverse effects	See Table A7_5_1_3-6a
4.2.2	Nature of adverse effects	Not relevant
4.3	**Test with reference substance**	Not performed
4.3.1	Concentrations	-
4.3.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test according to OECD Guideline 208 (Proposal for Updating Guideline 208, Draft Document, March 2005). Test was performed for 14 days in a growth chamber under controlled test conditions with three plant species: *Brassica napus, Glycine max, Avena sativa.* Effective concentrations were calculated based on fresh weight.
5.2	**Results and discussion**	
5.2.1	EC_{20}	Not described
5.2.2	EC_{50}	Based on fresh weight: *Brassica napus:* EC_{50} = 27.7 (25.3-30.2) mg a.i./kg soil; *Glycine max:* EC_{50} = 62.4 (2.65-30536) mg a.i./kg soil; *Avena sativa:* EC_{50} = 30.3 (27.7-33.2) mg a.i./kg soil
5.2.3	EC_{80}	Not described

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5.3	**Conclusion**	There is a clear dose-response relationship for all 3 plants. The validity criteria can be considered fulfilled according to the mentioned OECD guideline.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	No	

Section 7.5.1.3 Terrestrial plant toxicity

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]

Section 7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

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

	COMMENTS FROM ... *(specify)*
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 A7_5_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	-
Vehicle	Acetone
Concentration of vehicle	Adequate weights of Preventol CMK were diluted in 8-22 mL acetone.
Vehicle control performed	Yes
Other procedures	-

Table A7_5_1_3-2: Dilution water

Criteria	Details
Source	Not applicable, no dilution with water
Alkalinity / Salinity	
Hardness	
pH	
Oxygen content	
Conductance	
Holding water different from dilution water	

Table A7_5_1_3-3: Test plants

	Family	Species	Common name	Source (seed/plant)
Dicotyledonae	Brassicaceae	***Brassica napus***	Oilseed Rape	Source not mentioned in report
	Fabaceae	***Glycine max***	Soybean	
Monocotyledonae	Poaceae	***Avena sativa***	Oat	Source not mentioned in report

Table A7_5_1_3-4: Test system

Criteria	Details
Test type	Test was performed in a growth chamber under controlled test conditions.
Container type	Commercial plastic flower pots Size of pots: 12 cm diameter for *Avena sativa* or 14 cm diameter for *Brassica napus* and *Glycine max*
Seed germination potential	Germination rate in the study (solvent control): *Brassica napus*: 97 %, *Glycine max*: 100 %, *Avena sativa*: 93 %
Identification of the plant species	Each test unit was uniquely identified with study number, treatment and replicate number
Number of replicates	6 pots per treatment group were tested
Numbers of plants per replicate per dose	Each pot contained 5 seeds; in total 30 seeds per species and treatment group were tested.
Date of planting	2006-11-23
Plant density	Each pot contained 5 seeds; Size of pots: 12 cm diameter (for *Avena sativa*) or 14 cm diameter (for *Brassica napus* and *Glycine max*)
Date of test substance application	2006-11-22 to 2006-11-23
High of plants at application	The seeds were introduced manually into the treated soil.
Date of phytotoxicity rating or harvest	Shoot fresh weight: fresh weight of all survived plants of a pot (each pot is considered as a replicate) was determined 14 days after 50 % seedling emergence in the control; Germination: was checked daily until 50 % of the control plants had emerged. Further checks were done weekly. Mortality: The number of living and dead plants was recorded 14 days after 50 % seedling emergence in the control. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living. Phytotoxicity: Visual phytotoxicity (e.g. chlorosis, necrosis, abnormal growth) was recorded weekly according to EPPO Standard PP 1/135 (2) after 50 % seedling emergence in the control; Growth stages: Were reported according to BBCH-Monograph-Growth stages at day 14; Experimental completion date: 2006-12-11
Dates of analysis	2006-12-13 to 2006-12-15

Table A7_5_1_3-5a: Test conditions (Part 1)

Criteria	Details
Test type	Terrestrial plants, Seedling emergence and seedling growth test according to OECD 208
Method of application	Adequate weights of Preventol CMK were diluted in 8 to 22 mL acetone. Then the corresponding dilutions were added to 638.6 to 1756.2 g fine quartz sand (0.0125 mL acetone/1 g sand). After mixing with a laboratory mixer (10 minutes) to reach a homogenous distribution of the test item within the sand the treated sand was left overnight in a fume hood until the solvent had evaporated. The acetone control was treated in the same way as the test item groups (acetone: 22 mL, sand: 1756.2 g). Treatment of the control: The same amount of untreated quartz sand was added to the soil.
Application levels	Based on the results of the non-GLP range finder the following concentrations of Preventol CMK were prepared: *Brassica napus*: mg test item/kg soil dry weight (nominal) 0.96 2.40 6.00 15.0 37.5 93.8 234.4 *Glycine max*: mg test item/kg soil dry weight (nominal) 2.40 6.00 15.0 37.5 93.8 234.4 *Avena sativa*: mg test item/kg soil dry weight (nominal) 15.0 37.5 93.8 234.4

Table A7_5_1_3-5b: Test conditions (Part 2)

Criteria	Details
Dose rates	Dose rates: See above; Application order: 1. control, 2. test item (increasing concentrations)
Substrate characteristics	The soil was delivered and analysed by LUFA Speyer, Germany, Soil Type: LUFA 2.3 (USDA: sandy loam); Particle size: All particles ≤ 0.2 cm; C_{org}: 1.02 ± 0.15 %; pH: 6.2 ± 0.3
Watering of the plants	Not mentioned in the report
Temperature	The test plants were grown at 24.6 °C (range 20.3-29 °C). Measurement was done during the entire study with one measuring point every 15 minutes.
Thermoperiod	Not mentioned in the report
Light regime	Light regime: 16 hours light : 8 hours dark; Light intensity: 14562 (8690-20140) lux Measurement was done once a week during the entire study with 5 different measuring points for each species.
Relative humidity	56.5 % (range 35.7-80.7 %); Measurement was done during the entire study with one measuring point every 15 minutes.
Wind volatility	Not mentioned in the report
Observation periods and duration of test	Shoot fresh weight: fresh weight of all survived plants of a pot (each pot is considered as a replicate) was determined 14 days after 50 % seedling emergence in the control; Germination: was checked daily until 50 % of the control plants had emerged. Further checks were done weekly. Mortality: The number of living and dead plants was recorded 14 days after 50 % seedling emergence in the control. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living. Phytotoxicity: Visual phytotoxicity (e.g. chlorosis, necrosis, abnormal growth) was recorded weekly according to EPPO Standard PP 1/135 (2) after 50 % seedling emergence in the control; Growth stages: Were reported according to BBCH-Monograph-Growth stages at day 14; Test duration: 14 days

Table A7_5_1_3-5c: Test conditions (Part 3)

Criteria	Details
Pest control	Not applicable
Any other treatments and procedures	After development of the first true leaves, 1 g/l Flory 9 “Hydro” (Planta) and 0.022 g/l Fetrilon 13 % (Compo) were added to the water one to three times a week, depending on the development of the plants.

Table A7_5_1_3-6a: Effective phytotoxicity after test termination (Part 1)

		Germination		Mortality		Fresh weight			
	Treatment Group (mg test item/kg dry soil) (nominal)	(%)	Statistics	(%)	Statistics	(g)	SD	Effect (%)*	Statistics
Time of Evaluation** Species		Day 14		Day 14		Day 14			
Brassica napus	Control	90		0		10.44	1.55		
	Solvent Control	97		0		10.22	1.28		
	0.96	100	n.s.[3]	0	n.s.[3]	9.90	0.93	-3.1	n.s.[1]
	2.40	97	n.s.[3]	3	n.s.[3]	10.20	1.65	-0.2	n.s.[1]
	6.00	97	n.s.[3]	0	n.s.[3]	10.24	1.08	0.1	n.s.[1]
	15.0	97	n.s.[3]	0	n.s.[3]	8.75	1.17	-14.4	n.s.[1]
	37.5	93	n.s.[3]	0	n.s.[3]	3.07	0.62	-70.0	s.[1]
	93.8	73	s.[3]	45	s.[3]	0.04	0.03	-99.6	s.[1]
	234.4	0	s.[3]	-	-	-	-	-	-
Glycine max	Control	100		0		17.89	± 1.59		
	Solvent Control	100		0		17.19	± 1.05		
	2.40	100	n.s.[3]	3	n.s.[3]	15.99	± 2.52	-7.0	n.s.[2]
	6.00	93	n.s.[3]	0	n.s.[3]	12.88	± 3.23	-25.1	s.[2]
	15.0	100	n.s.[3]	0	n.s.[3]	14.71	± 1.90	-14.4	n.s.[2]
	37.5	100	n.s.[3]	0	n.s.[3]	12.07	± 1.30	-29.8	s.[2]
	93.8	93	n.s.[3]	0	n.s.[3]	8.31	± 0.85	-51.6	s.[2]
	234.4	10	s.[3]	0	n.s.[3]	0.08	± 0.03	-99.5	s.[2]
Avena sativa	Control	97		0		9.30	± 0.45		
	Solvent Control	93		0		8.57	± 0.90		
	15.0	87	n.s.[3]	0	n.s.[3]	7.28	± 2.02	-15.1	s.[2]
	37.5	93	n.s.[3]	0	n.s.[3]	3.28	± 0.89	-61.7	s.[2]
	93.8	97	n.s.[3]	0	n.s.[3]	0.24	± 0.06	-97.2	s.[2]
	234.4	10	s.[3]	33	n.s.[3]	0.01	± 0.00	-99.9	s.[2]

The results represent rounded values calculated on the exact raw data
SD Standard Deviation

* negative values indicate reduction compared to the solvent control
** days after 50 % seedling emergence in the control
- not evaluated, because there was no germination
[1] Multiple comparison Dunnett Test, α = 0.05, two sided
[2] Multiple comparison Bonferroni Welch-t-Test, α = 0.05, two sided
[3] Pairwise comparison Fisher Exact Test, α = 0.05, two sided
s. significant
n.s. not significant

Table A7_5_1_3-6b: Effective phytotoxicity after test termination (Part 2)

		Phytotoxicity (%)		Growth Stage (BBCH)
Time of Evaluation* Species	**Treatment Group (mg test item/kg dry soil) (nominal)**	**Day 7**	**Day 14**	**Day 14**
Brassica napus	Control	0	0	14
	Solvent Control	0	0	14
	0.96	0	1	14
	2.40	1	2	13-14
	6.00	0	0	14
	15.0	0	0	14
	37.5	20	23	12-13
	93.8	52	95	09-10
	234.4	-	-	-
Glycine max	Control	0	0	13-14
	Solvent Control	0	0	13-14
	2.40	0	3	13-14
	6.00	0	0	13-14
	15.0	0	0	13-14
	37.5	3	0	13-14
	93.8	28	11	13
	234.4	60	97	09
Avena sativa	Control	0	0	13
	Solvent Control	0	0	13
	15.0	2	2	13
	37.5	38	16	12
	93.8	63	83	10-11
	234.4	89	98	10

** days after 50 % seedling emergence in the control

Table A7_5_1_3-7: Summary of Effective concentrations *(based on fresh weight)*

		NOEC	LOEC	Statistical Analysis	EC_{25}	EC_{50}	Statistical Analysis
Species	**Confidence limit (c.l.)**	(mg a.i./kg soil)			(mg a.i./kg soil)		
Brassica napus		15.0	37.5	[1]	18.9	27.7	[3]
	lower 95 % c.l.				16.4	25.3	
	upper 95 % c.l.				21.0	30.2	
Glycine max		~~15.0~~ **2.4**	~~37.5~~ **6.0**	[2]	25.2	62.4	[3]
	lower 95 % c.l.				n.d.	2.65	
	upper 95 % c.l.				60.2	30536	
Avena sativa		< 15.0	15	[2]	19.4	30.3	[3]
	lower 95 % c.l.				16.8	27.7	
	upper 95 % c.l.				21.7	33.2	

[1] Multiple comparison Dunnett Test, $\alpha = 0.05$, two sided
[2] Multiple comparison Bonferroni Welch-t-Test, $\alpha = 0.05$, two sided
[3] Probit Analysis
n.d. not determined due to mathematical reasons

Section A7.5.2.1 **Annex Point IIIA 13.3**	**Reproduction study with earthworms or other soil non-target macro-organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	December 2011	
Evaluation of applicant's justification	[redacted]	
Conclusion		
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*	

Section A7.5.2.1 Annex Point IIIA 13.3	Reproduction study with earthworms or other soil non-target macro-organisms
Remarks	

Section A7.5.2.2 **Annex Point IIIA 13.3**	**Long-term tests with terrestrial plants**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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 FI	
Date	May 2008	
Evaluation of applicant's justification	[redacted]	
Conclusion		
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 A7.5.3.1.1 Acute oral toxicity on birds

Annex Point IIIA XIII
1.1

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1993): Preventol CMK: An acute oral LD_{50} with Bobwhite Quail. [redacted] 1993-02-19.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, FIFRA Subdivision E, § 71-1 "Acute oral LD_{50}" Toxic Substance Control Act (TSCA) ASTM Standard Practice (Draft 6) "Standard practice for conducting acute oral LD_{50} tests with avian species").
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Preventol CMK Technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis in the diet	Not stated
3.2	**Administration of the test substance**	See table A7_5_3_1_1-1
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference	-

Section A7.5.3.1.1 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

	substance	
3.4	**Testing procedure**	
3.4.1	Test organisms	See table A7_5_3_1_1-2
3.4.2	Test system	See table A7_5_3_1_1-3
3.4.3	Diet	See table A7_5_3_1_1-3
3.4.4	Test conditions	See table A7_5_3_1_1-4
3.4.5	Duration of the test	Acclimation: 7 weeks Fasting: 19 hours prior to dosing Dosing: the date of study initiation (November 24, 1992) Post-dosing observation: 14 days
3.4.6	Test parameter	Mortality, symptoms, body weight and feed consumption were monitored. Postmortem examinations were conducted on all mortalities and a percentage of birds sacrificed at study termination.
3.4.7	Examination/ Observation	See table A7_5_3_1_1-3
3.4.8	Statistics	The LD_{50} was calculated by Probit analysis. Bartlett´s test of equal variance was performed on body weight and feed consumption data to determine if test levels had unequal variances ($\alpha \leq 0.001$). Subsequent parametric analyses were conducted (if variances were not determined to be unequal) by one way ANOVA ($\alpha \leq 0.05$).
		4 RESULTS
4.1	**Limit Test / Range finding test**	No
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Applied concentrations	0, 62.5, 125, 250, 500, 1000 and 2000 mg a.i./kg bw
4.2.2	Effect data (Mortality)	Mortality occurred at concentrations ≥ 1000 mg a.i./kg bw. No male birds in the 2000 mg/kg level survived past day 1. Two females in the highest treatment group died on day 0 but the three remainig females survived to termination. Three birds died in the 1000 mg/kg level. One male died on day 0 and 2 females died on day 1. No further mortality was observed.

Section A7.5.3.1.1 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

		See also table A7_5_3_1_1-5
4.2.3	Body weight	See table A7_5_3_1_1-6
4.2.4	Feed consumption	See table A7_5_3_1_1-7
4.2.5	Concentration / response curve	Not given in the report
4.2.6	Other effects	Compound related symptoms were observed in birds treated at concentrations ≥ 250 mg/kg and included ataxia, hyporeactivity, immobility and pilorection (fluffed feathers). See also table A7_5_3_1_1-5.
4.3	**Results of controls**	There were no mortalities in the control group. All birds were normal in appearance and behaviour throughout the test period.
4.3.1	Number/ percentage of animals showing adverse effects	-
4.3.2	Nature of adverse effects	-
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Adult Bobwhite quail (*Colinus virginianus*) were used to evaluate the acute oral toxicity of Preventol CMK. Seven test levels of ten birds each (five males and five females) were given single oral doses of 0, 62.5, 125, 250, 500, 1000 and 2000 mg a.i./kg bw. Birds were observed for mortality and symptoms for 14 days. Body weight and feed consumption were monitored during the 14 days. The test complies with FIFRA Subdivision E, § 71-1 as well as those of the Toxic Substance Control Act (TSCA) and the ASTM Standard Practice (Draft 6) "Standard practice for conducting acute oral LD_{50} tests with avian species").
5.2	**Results and discussion**	Mortalities occurred in treatment groups reveiving ≥ 1000 mg a.i./kg bw. Treatment related symptoms were observed in levels ≥250 mg a.i./kg bw. No statistically significant decreases in body weight were observed and no overall significant decreases were detected in feed consumption.
5.2.1	LD_{50}	= 1449 mg a.i./kg bw
5.2.2	NOEC	= 125 mg a.i./kg bw
5.3	**Conclusion**	The mortality rate in the control was below 10% and the results can be considered as valid. See Table A7_5_3_1_1-8

Section A7.5.3.1.1 Acute oral toxicity on birds

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5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	No

Section A7.5.3.1.1 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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 A7_5_3_1_1-1: Method of administration of the test substance

Carrier/Vehicle	Details
Water	No
Organic carrier	No
concentration of the carrier [% v/v]	-
Other vehicle	Gelantine capsules (Eli Lilly and Company, Indianapolis, Indiana).
Function of the carrier / vehicle	-

Table A7_5_3_1_1-*2*: Test animals

Criteria	Details
Species/Strain	Bobwhite quail (*Colinus virginianus*)
Source	[redacted]
Age (in weeks), sex and initial body weight (bw)	Age: adult animals, 16 weeks old; Sex: males and females; Body weights ranged from 197 to 325grams at study initiation
Breeding population	No data
Amount of food	Throughout acclimation and testing all test birds were fed a game bird ration (Agway Gamebird Ration). Food and water were available ad libitum, prior to and throughout the study with the exception of the 19 hours immediately prior to dosing, during which the birds were fasted.
Age at time of first dosing	Age: adult animals, 16 weeks old
Health condition / medication	Not specified in the report

Table A7_5_3_1_1-3: Test system

Criteria	Details
Test location	Indoor, cages
Holding pens	Stainless steel cages (91 x 62 x 26 cm)
Number of animals	70 (35 males and 35 females)
Number of animals per pen [cm²/bird]	5 birds of a single sex
Number of animals per dose	One control groups, each with 5 males + 5 females, Six dose groups, each with 5 males + 5 females
Pre-treatment / acclimatisation	All test birds were acclimated to the caging and facilities for 7 weeks prior to the initiation of the study. The birds were fasted for approximately 19 h prior to dosing. During acclimation all birds were observed daily.
Diet during test	Food (Agway Gamebird Ration, contaminant analysed) and water were available ad libitum throughout the study except the fasting period of 19 hours prior to dosing.
Dosage levels (of test substance)	0, 62.5, 125, 250, 500, 1000 and 2000 mg a.i./kg bw
Replicate/dosage level	Six dose groups were investigated, each dose group with 5 males and 5 females;
Feed dosing method	Birds received a single oral dose via gelatine capsules.
Dosing volume per application	2 gelatine capsules per bird, dosages were adjusted for percent acive ingredient based on treatment level and weight of bird.
Frequency, duration and method of animal monitoring after dosing	Feed consumption for each group of 5 birds was recorded daily. Observations for mortality and clinical symptoms were recorded approximately 1, 2.5 and 5 hours post-dosing on day 0. Throughout the remainder of the study, birds were observed at least twice daily (post-dosing observation for 14 days), except on weekends and holidays when only one observation per day was made. Postmortem examinations were conducted at study termination.
Time and intervals of body weight determination	Individual body weights were measured at the initiation of the study (day 0) and on days 7 and 14.

Table A7_5_3_1_1-4: Test conditions (housing)

Criteria	Details
Test temperature	23 ± 1°C.
Shielding of the animals	No data
Ventilation	No data
Relative huminity	40 %
Photoperiod and lighting	8/16 hour light/dark cycle

Table A7_5_3_1_1-5: Mortalities and toxic symptoms observed in Bobwhite quail orally dosed with Preventol CMK

Dosage [mg a.i./kg b.w.]	Dead	Dosed	Exhibiting toxic signs	Observations *
Control	0	10	0	None
62.5	0	10	0	None
125	0	10	0	None
250	0	10	2	Ataxia (2)
500	0	10	2	Ataxia (2) Pilorection (2)
1000	3	10	7	Ataxia (3) Hyporeactivity (6) Immobility (5)
2000	7	10	9	Ataxia (4) Hyporeactivity (4) Immobility (4)

* Numbers in parentheses denote the number of birds exhibiting the symptom

Table A7_5_3_1_1-6: Average body weights of Bobwhite quail orally dosed with Preventol CMK

Dosage [mg a.i./kg b.w.]		Average body weight [g]		
		Initiation (day 0)	Day 7	Termination (day 14)
Control	Female	231.4	240.4	245.2
	Male	229.8	232.6	235.4
62.5	Female	232.8	240.6	239.4
	Male	240.6	246	246.6
125	Female	229.6	238.8	239.6
	Male	238.8	246.8	247.6
250	Female	238.8	240	243
	Male	234.2	238.8	241.8
500	Female	221.2	228.4	230.4
	Male	253.8	258.2	264
1000	Female	239.8	240.3	247.7
	Male	248.6	259.0	267.0
2000	Female	226.2	216.3	230.3
	Male	245.8	-	-

Table A7_5_3_1_1-7: Mean daily feed consumption of Bobwhite quail orally dosed with Preventol CMK

Dosage [mg a.i./kg bw]	Feed consumption [g/bird/day]													
	day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	12.1	19.1	19.5	16.3	20.1	13.8	16.8	13.3	27.8	26.9	22.3	26.5	15.8	17.6
62.5	12.3	31.3	21.4	18.6	18.1	17.1	17.8	12.4	38.0	27.2	22.7	19.5	14.7	18.6
125	11.1	28.1	24.1	19.0	19.9	14.5	17.7	15.4	32.1	20.2	22.0	20.5	13.6	18.8
250	7.2	26.8	19.5	16.5	19.5	13.9	16.3	14.1	26.5	21.1	18.9	21.0	13.2	16.0
500	6.7	24.2	18.3	16.6	21.2	13.9	18.6	16.0	37.4	22.2	18.0	22.3	13.4	20.0
1000	4.1	38.9	28.0	20.9	23.8	16.6	18.8	20.3	52.4	28.3	24.1	24.8	15.8	27.7
2000	1.0	10.0	24.7	16.7	19.0	13.3	15.0	14.3	43.7	28.0	24.0	21.0	14.3	31.3

Table A7_5_3_1_1-8: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	**Not fulfilled**
Mortality of control animals <10%	**X**	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1993): Preventol CMK: A subacute dietary LD_{50} with Bobwhite Quail. [redacted] 1993-02-19.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Lanxess Deutschland GmbH	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, § 71-2, October 1982 "Avian subacute dietary LC_{50}"; ASTM Standard E857-81: "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species"	
2.2	**GLP**	Yes	
2.3	**Deviations**	Deviations: No deviations from EPA § 71-2	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Preventol CMK Technical	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis in the diet	Gas chromatograph (GC) equipped with a flame ionisation detector (FID) A Report about analytical methods and analytical results (ABC Laboratories, Report No. 40468, Date: 1993-02-09) is attached to the original report.	
3.2	**Administration of the test substance**	Birds were exposed for five days to a nominal dietary concentrations of 388, 648, 1080, 1800, 3000 and 5000 mg a.i./kg feed. See table A7_5_3_1_2-2	
3.3	**Reference substance**	No	
3.3.1	Method of analysis	-	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

	for reference substance	
3.4	**Testing procedure**	
3.4.1	Test organisms	See table A7_5_3_1_2-1
3.4.2	Test system	See table A7_5_3_1_2-2
3.4.3	Diet	See table A7_5_3_1_2-2
3.4.4	Test conditions	See table A7_5_3_1_2-3
3.4.5	Duration of the test	Pre-exposure: 3-day observation period prior to compound administration Exposure: 5-day observation period when treated diets were administered Recovery: 3-day post-exposure observation period after treated diets had been removed
3.4.6	Test parameter	Mortality, clinical symptoms, body weight changes, feed consumption Postmortem examinations were conducted on all mortalities and a percentage of birds sacrificed at study termination.
3.4.7	Examination/ Observation	See table A7_5_3_1_2-2
3.4.8	Statistics	Since there were less than 50 % mortality at the highest concentration tested, an LC_{50} was not calculated and an estimation of the LC_{50} value was made by a visual inspection of the mortality data. Bartlett´s test of equal variance was performed on body weight and feed consumption data to determine if test levels had unequal variances ($\alpha \leq 0.001$). Subsequent parametric analyses were conducted (if variances were not determined to be unequal) by one way ANOVA. If ANOVA indicated treatment means were significantly different from controls ($\alpha \leq 0.05$), the means of the treatment groups were compared to control means using a one-tailed Dunnett´s test.
		4 RESULTS
4.1	**Limit Test / Range finding test**	No
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Applied concentrations	nominal dietary concentrations: 388, 648, 1080, 1800, 3000 and 5000 mg a.i./kg diet measured concentrations: 280, 491, 884, 1530, 2420 and 4250 mg a.i./kg diet

X

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

		two controls (untreated feed and untreated feed containing equivalent amounts of corn-oil as carrier and acetone as solvent) Measured concentrations represent a range of 72 - 85% recovery of nominal concentration. The compound did not prove to be highly stable at room temperature over the 5-day exposure period. Day 5 measurements ranged from 37 - 50% of the initial measured concentrations. All observations will refer to measured concentrations.
4.2.2	Effect data (Mortality)	Treatment-related mortalities did not occur in any of the treatment levels. One bird in the 491 mg a.i./kg diet treatment group was found dead on day 1 but this was not determined to be treatment related as no other mortalities occurred in the study. No birds in any treatment level exhibited any symptoms of toxicity or intoxication.
4.2.3	Body weightt	The 1530 mg a.i./kg diet group remained significantly lighter throughout the study and was not considered e treatment effect. The 4250 mg a.i./kg diet did show significantly reduced body weights at study termination (day 8). See Table A7_5_3_1_2-5
4.2.4	Feed consumption	As only one feed consumption measurement per level per day was generated, replication is lacking and a statistical analysis on daily feed consumption was not possible. No statistical different reduction in feed consumption compared to control was observed during the exposure period. On contrary, during the post-exposure period, feed consumption was significantly reduced compared to controls (Dunnett´s test, $\alpha \leq 0.05$) in all treatment levels except 2420 mg a.i./kg diet. See Table A7_5_3_1_2-6
4.2.5	Concentration / response curve	Not given in the report
4.2.6	Other effects	Postmortem examination revealed no gross lesions or unusual observations. The bird in the 491 ppm treatment group that died exhibited autolytic signs such as fluid-filled and gas-filled intestines.
4.3	**Results of controls**	No mortalities were observed in the control. All birds of the control group were normal in appearance and behaviour throughout the test period. No significant differences in body weight and feed consumption between the control groups occurred and thus, control groups were combined for subsequent statistical analyses.
4.3.1	Number/ percentage of animals showing adverse effects	-
4.3.2	Nature of adverse effects	-
4.4	**Test with reference substance**	Not performed

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a five-day dietary toxicity study according to US-EPA FIFRA Guideline 71-2, 10 days old bobwhite quails (*Colinus virginianus;* Galliformes: Megapodiidae) were fed measured dietary levels of 280, 491, 884, 1530, 2420 and 4250 mg a.i./kg diet. Two control groups received untreated feed (control 1) and an amount of carrier and solvent in their diet equivalent to that used in the treated diets (control 2).	X
		After treatment, the condition of the quails was monitored on untreated feed for further three days. Mortality, clinical symptoms, body weight changes, feed consumption were observed during the 5-day exposure and the 3-day post exposure period. Postmortem examinations were conducted on all mortalities and a percentage of birds sacrificed at study termination.	
5.2	**Results and discussion**	Birds fed with diet levels up to 4250 mg Preventol CMK/kg diet showed no mortalities or signs of intoxication from ingestion of the compound.	
		No statistically significant depression of body weight or growth was noted during the exposure period. The 4250 mg a.i./kg diet group was significantly lighter than the control group at the end of the recovery period. This was supported by significantly reduced growth and feed consumption in this group during the recovery period.	
		There were no significant differences in feed consumption during the exposure period. Significant differences in feed consumption appeared during the recovery period. These effects may represent cage-to-cage variation rather than treatment effects. There is the possibility that reduced feed consumption at most levels during this period represents a preference for Preventol that developed. When birds were switched to control 2 feed, the different taste resulted in less food consumption. It is also possible that there is some delayed reaction to the compound that surfaced during the recovery period.	
5.2.1	LC_{50}	LC_{50} > 4250 mg a.i./kg diet	X
5.2.2	NOEC	NOEC = 4250 mg a.i./kg diet (based on exposure period)	X
5.3	**Conclusion**	One of the three validity criteria for short-term avian toxicity test according to OECD Guideline 205 is not fulfilled (see also Table A7_5_3_1_2-8):	
		The day 5 measurements ranged from 37 - 50% of the initial measured concentrations, i.e. below 80%. Thus, all observations will refer to initial measured concentrations.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

	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 A7_5_3_1_2-1: Test animals

Criteria	Details
Species/Strain	Bobwhite quail (*Colinus virginianus*)
Source	[redacted]
Age, sex and initial body weight (bw)	Age: 7 days at beginning of 3-days pre-exposure period Sex: unknown Body weight of birds in the control group: 26 ± 2 g on day 0
Breeding population	Bobwhite quail eggs were obtained from a quail farm and hatched at the study site. Upon hatch, the chicks were housed in galvanised steel brooders and were acclimatised for 7 days prior to being randomised into test groups.
Amount of food	Water and feed were provided *ad libitum*.
Age at time of first dosing	Age: 10 days
Health condition / medication	Not specified in the report

Table A7_5_3_1_2-2: Test system

Criteria	Details
Test location	Indoor, steel cages
Holding pens	Galvanised steel brooders (91 x 71 x 23 cm high)
Number of animals	80 (60 for dose groups, 20 for control groups (10 for control 1: raw feed, 10 for control 2: vehicle/solvent control))
Number of animals per pen [cm²/bird]	10 birds of unknown sex (646.1 cm²/bird)
Number of animals per dose	Two control groups and six test concentrations (nominal dietary concentrations: 388, 648, 1080, 1800, 3000 and 5000 mg a.i./kg diet). Each treatment or control group contained ten birds.
Pre-treatment / acclimatisation	Eggs hatched at the study site. Quails were acclimatised for 7 days and then randomised into the brooders with 10 birds per cage. Once test assignments were complete, all test levels were given untreated control feed (control 1) and feed consumption was monitored for three days prior to test initiation.
Diet during test	Food (Tekland JQ-15 Quail Starter) and water (Kansas city municipal water) were available ad libitum. Diet was prepared with appropriate amounts of Preventol CMK, corn oil (carrier) and acetone (solvent) to obtain nominal concentration of 388, 648, 1080, 1800, 3000 and 5000 mg a.i./kg feed. Control 2 diets were prepared with the same amount of carrier and solvent as the treated diets. Control 1 birds received raw feed. Following the 3-day pre-exposure period the treated diets were administered to treatment level birds. After 5 days of exposure, treatment level birds were given a control diet (control 2) for a 3-day recovery period.
Dosage levels (of test substance)	Birds were exposed for five days to nominal dietary concentrations of 388, 648, 1080, 1800, 3000 and 5000 mg a.i./kg diet, corresponding to measured concentrations of 280, 491, 884, 1530, 2420 and 4250 mg a.i./kg diet.
Replicate/dosage level	Six dose groups were investigated
Feed dosing method	Orally by feed
Dosing volume per application	Food was available *ad libitum*
Frequency, duration and method of animal monitoring after dosing	Feed consumption for each level was recorded daily. Observations for mortality and clinical signs of toxicity were recorded approx. 1, 3 and 6 hours after diet administration, and twice daily throughout the remainder of the study, except on weekends when only one observation per day was made.
Time and intervals of body weight determination	Body weights were recorded within 24 hours of study initiation, at study initiation, on day 5 and at study termination (day 8).

Table A7_5_3_1_2-3: Test conditions (housing)

Criteria	Details
Test temperature	Internal temperature gradient from approximately 22.2 - 37.8°C
Shielding of the animals	No data
Ventilation	No data
Relative humidity	32 %
Photoperiod and lighting	16/8 hour light/dark cycle with a 40 minutes dawn/dusk cycle

Table A7_5_3_1_2-4: Mortalities and toxic symptoms observed in the Preventol CMK Bobwhite quail dietary LC_{50} test

Measured dietary concentration [mg a.i./kg diet]	Dead	Dosed	Exhibiting toxic signs	Observations
Control 1	0	10	0	None
Control 2	0	10	0	None
280	0	10	0	None
491	1	10	0	None
884	0	10	0	None
1530	0	10	0	None
2420	0	10	0	None
4250	0	10	0	None

Table A7_5_3_1_2-5: Mean body weight and growth data for Bobwhite quails in the Preventol CMK dietary LC_{50} test

Measured dietary concentration [mg a.i./kg diet]	Mean body weight ± Standard deviation (SD) [g]			Growth [g]	
	Day 0	Day +5	Day +8	Initiation to day 5	Day 6 to termination
Control [a]	26 ± 2	41 ± 4	54 ± 6	15 ± 2	13 ± 3
280	25 ± 3	41 ± 5	53 ± 7	15 ± 2	12 ± 3
491	25 ± 2	40 ± 4	52 ± 5	15 ± 2	13 ± 1
884	24 ± 2	39 ± 4	50 ± 6	14 ± 2	11 ± 1 [b]
1530	23 ± 3 [b]	37 ± 4 [b]	48 ± 6 [b]	14 ± 2	11 ± 2 [b]
2420	24 ± 2	40 ± 2	52 ± 3	16 ± 2	12 ± 1
4250	25 ± 3	38 ± 4	49 ± 4 [b]	13 ± 1	11 ± 1 [b]

[a] Control values represent the means of Control 1 and control 2
[b] Statistically significant difference (Dunnett´s; $p \leq 0.05$)

Table A7_5_3_1_2-6: Feed consumption for Bobwhite quail in the Preventol CMK dietary LC_{50} test

Measured dietary concentration [mg a.i./kg diet]	Feed consumption [g/bird/day]		
	Pre-treatment day -1 to day -2	Treatment day 0 to day 5	Post-treatment day 6 to day 8
Control [a]	5.6 ± 0.5	5.9 ± 1.1	8.5 ± 0.4
280	5.5 ± 0.7	6.1 ± 0.9	7.7 ± 0.2 [b]
491	5.7 ± 0.8	5.8 ± 0.5	7.7 ± 0.4 [b]
884	5.6 ± 0.6	5.6 ± 0.4	7.6 ± 0.6 [b]
1530	4.6 ± 0.3	5.4 ± 0.3	7.3 ± 0.3 [b]
2420	5.1 ± 0.1	5.9 ± 0.4	8.0 ± 0.2
4250	5.8 ± 0.7	5.3 ± 0.3	7.1 ± 0.1 [b]

[a] Control values represent the means of Control 1 and control 2
[b] Statistically significant difference (Dunnett´s; $p \leq 0.05$)

Table A7_5_3_1_2-7: Validity criteria for short-term avian toxicity test according to OECD Guideline 205

	Fulfilled	Not fulfilled
Mortality of control animals < 10 %	**X**	
Test substance concentration > 80 % of nominal concentration throughout the dosing period		**X**
Lowest treatment level causing no compound-related mortality or other observable toxic effects	**X**	

Section A7.5.3.1.3 **Annex Point IIIA 13.1.3**	**Effects on birds: Effects on reproduction**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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 FI	
Date	May 2008	
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 A7.5.4.1 **Annex Point IIIA 13.3**	**Acute toxicity to honeybees and other beneficial arthropods, for example predators**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	December 2011	
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		

Section A7.5.5 (01) Bioconcentration, terrestrial / further studies

Annex Point IIIA 13.3

Official use only

		1 REFERENCE
1.1	**Reference**	Fàbregas, E. (2007): p-Chloro-m-cresol - Calculation of the Bioconcentration Factor in Earthworms ($BCF_{earthworm}$). Dr. Knoell Consult GmbH, Leverkusen, Germany, Report No. KC-BCF-06/07, date: 2007-05-30 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not applicable, calculation method
2.2	**GLP**	Not applicable
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	p-Chloro-m-cresol
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Not applicable
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Log K_{ow} = 3.02; K_{ow} = 1047.13 (Ref.: Reusche, 1991)
3.1.5	Radiolabelling	--
3.1.6	Method of analysis	--
3.2	**Reference substance**	--
3.2.1	Method of analysis for reference substance	-
3.3	**Testing/estimation procedure**	
3.3.1	Test system/ performance	Not applicable
3.3.2	Estimation of bioconcentration	The bioconcentration factor in earthworm was calculated using the equation 82d of the Technical Guidance Document (EU, 2003). The bioconcentration factor can be measured experimentally directly. However, a specific test guideline for such a test is not available at the time being. The assessment of the $BCF_{earthworm}$ is necessary for chemicals which are, based on the use pattern, considered to enter the soil compartment.

Section A7.5.5 (01) Bioconcentration, terrestrial / further studies

Annex Point IIIA 13.3

Another possibility is the estimation of $BCF_{earthworm}$ from log K_{ow}. When measured data on bioconcentration in earthworms is not available the BCF will have to be estimated. For organic chemicals, the main route of uptake into earthworms will be via the interstitial water.

Bioconcentration can be described as a hydrophobic partitioning between the pore water and the phases inside the organism and can be modelled according to the following equation as described by Jager (1998):

$BCF_{earthworm} = (0.84 + 0.012K_{ow})/RHO_{earthworm}$

where for $RHO_{earthworm}$ by default a value of 1 ($kg_{wwt}.L^{-1}$) can be assumed.

Jager (1998) has demonstrated that this approach performed very well in describing uptake in experiment with earthworms kept in water. For soil exposure, the scatter is larger and the experimental BCFs are generally somewhat lower than the predictions by the model. The reasons for this discrepancy are unclear but may include experimental difficulties (a lack of equilibrium or purging method) or an underestimated sorption*.

The model was supported by data with neutral organic chemicals in soil within the range log K_{ow} 3-8 and in water-only experiments from 1-6. An application range of 1-8 is advised and it is reasonable to assume that extrapolation to lower K_{ow} values is possible. The model could also be used for chlorophenols when the fraction in the neutral form was at least 5% and when both sorption and BCF are derived from the K_{ow} of the neutral species. The underlying data are however too limited to propose this approach in general for ionised chemicals.

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*According to certain studies some soil ingesting organisms may accumulate chemical substances not only from the soil pore water but also directly (possibly by extraction in the digestive tract) from the fraction of the substance adsorbed onto soil particles. This may become important for strongly adsorbing chemicals, e.g. those with a $\log K_{ow} > 3$. For these compounds the total uptake may be underestimated. In other studies however it has been shown that soil digesters virtually only bioaccumulate the substance via the pore water, i.e. bioconcentrate chemical substances from the soil pore water. At present the latter process can be modelled by use of the equilibrium partitioning theory (cf. also Section 3.5 of TGD).

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	--
4.1.2	Lipid content	--
4.1.3	Concentrations of test material during test	--
4.1.4	Bioconcentration factor ($BCF_{earthworm}$)	Bioconcentration factor in earthworm is not based on measurements

Section A7.5.5 (01) Bioconcentration, terrestrial / further studies

Annex Point IIIA 13.3

4.1.5	Uptake and depuration rate constants	--
4.1.6	Depuration time	--
4.1.7	Metabolites	--
4.1.8	Other Observations	--
4.2	**Estimation of bioconcentration**	The obtained $BCF_{earthworm}$ by this method is 13.41.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The $BCF_{earthworm}$ of CMK was estimated using the QSAR-approach as recommended in the Technical Guidance Document on Risk Assessment (EU, 2003) based on a measured log K_{ow} value. If measured $BCF_{earthworm}$ values are not available, the BCF for earthworms can be predicted from the relationship between K_{ow} and $BCF_{earthworm}$. For organic chemicals, the main route of uptake into earthworms will be via the interstitial water. Bioconcentration can be described as a hydrophobic partitioning between the pore water and the phases inside the organism and can be modelled according to the following equation as described by Jager (1998): $BCF_{earthworm} = (0.84 + 0.012K_{ow})/RHO_{earthworm}$
5.2	Results and discussion	Considering a log K_{ow}-value of 3.02 or K_{ow} of 1047.13 which was obtained in a previously performed experimental study, the calculated $BCF_{earthworm}$-value of CMK was 13.41.
5.3	**Conclusion**	Based on a log K_{ow} value of 3.02, obtained from an experimental study, a $BCF_{earthworm}$ of 13.41 is obtained. This value indicates a low bioaccumulation potential of CMK in earthworms.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	--

Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2008
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.6 **Annex Point IIIA 13.3**	**Effects on other terrestrial non-target organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	May 2008	
Evaluation of applicant's justification	[redacted]	
Conclusion		
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 7.5.7.1 **Annex Point IIIA 13.3**	**Effects on mammals: acute oral toxicity, short term toxicity, effects on reproduction**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
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	May 2009	
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		