CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: p-chloro-m-cresol (CMK)

EC Number: 200-431-6

CAS Number: 59-50-7

Index Number: 604-014-00-3

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	4-Chloro-3-methylphenol
EC number:	200-431-6
CAS number:	59-50-7
Annex VI Index number:	604-014-00-3
Degree of purity:	***> 99.8%***
Impurities:	**See confidential information**

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 4* - H302 Acute Tox. 4* - H312 Eye Dam. 1 - H318 Skin Sens 1 - H317 Aquatic acute 1 - H400
Current proposal for consideration by RAC	Acute Tox. 4 - H302 ~~Acute Tox. 4 - H312~~ Skin irrit. 2; H315 STOT SE 3 H335 Skin Sens 1B - H317 Aquatic acute 1 - H400 (existing classification

	coming from translation of DSD: data presented for confirmation of the category of hazard) Aquatic chronic 3- H412 M-factor acute : 1
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4 - H302 Skin irrit. 2 - H315 Eye Dam. 1 - H318 STOT SE 3 - H335 Skin Sens 1B - H317 Aquatic acute 1 - H400 Aquatic chronic 3- H412 M-factor acute : 1

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	No classification			conclusive but not sufficient for classification
2.2.	Flammable gases	Not considered			conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not considered			conclusive but not sufficient for classification
2.4.	Oxidising gases	Not considered			conclusive but not sufficient for classification
2.5.	Gases under pressure	No classification			conclusive but not sufficient for classification
2.6.	Flammable liquids	Not considered			conclusive but not sufficient for classification
2.7.	Flammable solids	No classification			conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	No classification			conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not considered			conclusive but not sufficient for classification
2.10.	Pyrophoric solids	No classification			conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	No classification			conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	No classification			conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not considered			conclusive but not sufficient for classification
2.14.	Oxidising solids	No classification			conclusive but not sufficient for classification
2.15.	Organic peroxides	No classification			conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral	Acute Tox. 4 - H302	-	Acute Tox. 4 - H302	
	Acute toxicity - dermal	No classification	-	Acute Tox. 4 - H312	conclusive but not sufficient for classification

	Acute toxicity - inhalation	No classification	-	No classification	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin irrit. 2 - H315	-	No classification	
3.3.	Serious eye damage / eye irritation	Eye Dam. 1 - H318	-	Eye Dam. 1 - H318	
3.4.	Respiratory sensitisation	No classification	-	No classification	Data lacking
3.4.	Skin sensitisation	Skin Sens 1B - H317	-	Skin Sens 1 - H317	
3.5.	Germ cell mutagenicity	Not considered	-	No classification	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not considered	-	No classification	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not considered	-	No classification	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	STOT SE 3 - H335	-	No classification	
3.9.	Specific target organ toxicity – repeated exposure-	Not considered	-	No classification	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not considered	-	No classification	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic acute 1 - H400 Aquatic chronic 3- H412	M-factor acute : 1	Aquatic acute 1 - H400	
5.1.	Hazardous to the ozone layer	No classification	-	No classification	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Dgr
Hazard statements: H410
Precautionary statements: not harmonised

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

CMK is an active Biocide substance in the meaning of Regulation EC 528/2012.

The harmonised classification of CMK was first introduced in the 29th ATP (Directive 2008/58/EC). It has not been modified since then.

New data available from the Biocide risk assessment and from literature now demonstrate the need to revise environmental classification.

2.2 Short summary of the scientific justification for the CLH proposal

Because acute toxicity 4 are translation from Directive 67/548/EEC, FR proposes to present the data on these classifications. Based on the available data presented below, it is proposed to confirm the **Acute toxicity 4 after oral** administration but to withdraw the on **after dermal** administration. Skin sensitization for which the subcategory is proposed in light of human and animal data. There is a proposal to include a classification for STOT SE based on the observed irritation of respiratory system, skin irritation based on animal data and aquatic chronic classification.

Regarding all available aquatic toxicity data, fish are the most sensitive species for acute and chronic effects. These results are used to classify the active substance CMK.

Based on the fact that the current classification for **Aquatic Acute 1** is based on translation from Directive 67/548/EEC, FR proposes to present the data confirming this classification. Considering that the 96h-LC_{50} = 0.92 mg/L value was obtained for *Oncorhynchus mykis* is lower than 1 mg/L, CMK meets the criteria for classification as **Aquatic Acute 1** for environmental hazard according to CLP criteria. As this value is within the range of 0.1- 1.0 mg/L, an **M-factor of 1** is allocated.

Considering that CMK is rapidly degradable and that the 28d-NOEC = 0.15 mg/L value obtained for *Oncorhynchus mykis* is within the range of 0.1- 1.0 mg/L, CMK meets the criteria for classification as **Aquatic Chronic 3** for environmental hazard according to CLP criteria.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

The classification of CMK is harmonised in Annex VI of CLP under the index number 604-014-00-3 as follows:

Classification according to Regulation (EC) No 1272/2008 (CLP)

Class of danger	Acute Tox. 4*	
	Eye Dam. 1	
	Skin Sens 1	
	Aquatic acute 1	
Hazard Statement	H302	Harmful if swallowed.
	H312	Harmful in contact with skin.
	H318	Causes serious eye damage.
	H317	May cause an allergic skin reaction.
	H400	Very toxic to aquatic organisms (M factor = 1).

*minimal classification obtained from conversion of DSD classification

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

2.4 Current self-classification **and labelling**

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Current self-classification and labelling do not differ from the harmonised classifications and labelling for the environment. For human health, some notifiers propose a classification as skin Corrosive cat. 1C (355 notifiers).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

CMK is an active Biocide substance in the meaning of Regulation EC 528/2012. In accordance with Article 36(2) of the CLP Regulation, CMK shall be subjected to harmonise classification and labeling for all endpoints. CMK already has a harmonized classification but available data demonstrate the need to revise some categories. Acute HH and aquatic toxicities are translations and are therefore presented in the dossier, together with skin sensitization for which the subcategory is proposed. There is a proposal to include a classification for STOT SE, skin irritation and aquatic chronic classification.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

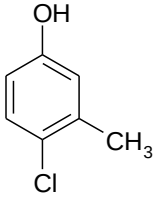
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	200-431-6
EC name:	Chlorocresol
CAS number:	59-50-7
CAS name:	Phenol, 4-Chloro-3-methyl-
IUPAC name:	4-Chloro-3-methylphenol
CLP Annex VI Index number:	
Molecular formula:	C_7H_7ClO
Molecular weight range:	142.6 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
4-Chloro-3-methylphenol	-	>99.8%	-

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential information			

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	nearly white solid pellets	Kraus, 2006a	Visual inspection Purity: min. 99.8%
Melting/freezing point	Mp = 64.2 °C	Erstling, 2007	EC method A.1 (DTA) Purity: min. 99.8%
Boiling point	Bp = 242 °C	Erstling, 2008	EC method A.2 (Siwoloboff)) Purity: min. 99.8%
Relative density	1.335 at 20 °C	Erstling, 2001a	EC method A.3 Displacement method Purity: min. 99.8%
Vapour pressure	1.4×10^{-03} Pa at 20 °C 6.0×10^{-03} Pa at 25 °C 3.8 Pa at 50 °C	Wielpütz, 2008	EC method A4 (effusion method) Purity: min. 99.8%
Surface tension	Results at 20 °C: 6.05×10^{-05} $Pa\times m^3\times mol^{-1}$ (pH 5) 5.87×10^{-05} $Pa\times m^3\times mol^{-1}$ (pH 7) 4.87×10^{-05} $Pa\times m^3\times mol^{-1}$ (pH 9)	Wielpütz, 2008 Erstling, 2001b	EC method A.5 Purity: min. 99.8%
Water solubility	<u>Results at pH 5:</u> 2.5 g/L at 10°C 3.3 g/L at 20°C 4.5 g/L at 30°C <u>Results at pH 7:</u> 2.6 g/L at 10°C 3.4 g/L at 20°C 4.6 g/L at 30°C <u>Results at pH 9:</u> 3.1 g/L at 10°C 4.1 g/L at 20°C 5.5 g/L at 30°C	Erstling, 2001b	EC method A.6 (flask method) Purity: min. 99.8%
Partition coefficient n-octanol/water	Log Pow = 3 at 22°C and pH = 6.4	Erstling, 2001c Reusche, 1991	EC method A.8 (HPLC) OECD guideline 107 Purity: min. 99.8%
Flash point	Not relevant		
Flammability	CMK is not highly flammable.	Heitkamp, 2006	EC method A.10 Purity: min. 99.8%
Explosive properties	solid material does not present any risk for explosion	Kraus, 2006b	Based on scientific judgement

Self-ignition temperature	CMK does not undergo spontaneous combustion.	Heitkamp, 2006	EC method A.16 Specification: min. 99.8%
Oxidising properties	active substance does not have oxidising properties	Kraus, 2006c	Based on scientific judgement
Granulometry	< 1 µm: <0.006% > 2000 µm : 91.7 %	Erstling, 2008	
Stability in organic solvents and identity of relevant degradation products	Not relevant		
Dissociation constant	pK = 9.4 ± 0.1 at 20 °C	Reusche, 1991	OECD guideline 112 Purity: min. 99.8%
Viscosity	Not relevant		

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

CMK is used as a biocidal product.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1.1 Summary and discussion of physico-chemical properties

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A10 (flammability)	negative	Test provided	Heitkamp, 2006
EEC A 16 (auto-flammability)	negative	Test provided	Heitkamp, 2006
EEC A14 (explosivity)	Negative	Theoretical statement	Kraus, 2006b
EEC A 17 (oxidizing properties)	negative	Theoretical statement	Kraus, 2006c

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

CMK is not highly flammable, it does not liberate gases in hazardous amounts in contact with water, it does not deliver indications of pyrophoric properties and does not undergo spontaneous combustion.

Based on scientific judgement it is certified that the structural formula CMK contains no oxidising groups or other chemically instable functional groups in its molecular backbone. Thus the active substance is incapable of rapid decomposition with evolution of gases or release of heat, and may not react exothermically with a combustible material, i.e. the solid material does not present any risk for explosion and does not have oxidising properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not considered in this dossier

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Route	Method Guideline	Species, Strain, Sex, No/group	Dosage, Duration of exposure	Value LD50/LC50	Reference
Oral	LD_{50} test (Limit test) EEC method B.1	Rat, Wistar ♂+♀, 5/sex/group	2000 mg/kg bw	< 2000 mg/kg	Bomhard, 1988a
Oral	LD_{50} test No guideline Non-GLP	Rat, Wistar II ♂, 10/group	1000, 1500, 2000, 3100, 5000 mg/kg bw (♂)	1830 mg/kg (♂)	Bomhard, 1978 and Löser, 1992
Dermal	LD_{50} test OECD 402	Rat, Wistar ♂+♀, 6/sex/dose	0, 2000 (♀), 5000 mg/kg 24 h	> 2000 mg/kg (♀) > 5000 mg/kg (♂)	Sturdivant, 1999
Dermal	LD_{50} test No guideline, but ≅ OECD 402	Rabbit, NZW ♂+♀, 6/sex/dose	0, 250, 500, 1000, 5000 mg/kg, 24 h	> 5000 mg/kg	Rutter *et al.* 1979
Inhalation	LC_{50} test OECD 403	Rat, Wistar ♂+♀, 5/sex/group	0, 1337, 2871 mg/m³, 4 h	> 2871 mg/m³	Pauluhn, 2003

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Three studies for acute oral toxicity in the rat were conducted with the test substance p-chloro-m-cresol (CMK).

Reference: Bomhard E. (1988), Preventol CMK Untersuchung zur akuten oralen Toxizität an männlichen und weiblichen Wistar-Ratten Bayer AG, Institut für Toxikologie, Wuppertal, Germany - Report No. 17062, 1988-08-18 (unpublished)

Guidelines: 84/449/EC, EEC Method B.1
GLP standards: Yes
Deviations: Yes
- acclimatisation period was only 4 days
- only one dose was tested in spite of mortality

Study acceptable: Yes.
Test system: p-Chloro-m-cresol, Batch number: 792, purity: 99.9%

Method:
Wistar rats per sex received 2000 mg CMK/kg bw as a solution in polyethyleneglycol 400 by single-dose oral gavage. In-life observations were made frequently on the day of treatment and twice each working day and once daily on weekends throughout the two-week observation period. Surviving animals were weighed before treatment, after one week and at study termination. Pathological-anatomical examinations were performed on all animals.

Results:
All animals showed slight to moderate signs of toxicity beginning 15 minutes after application and lasting up to 5 days. The clinical signs observed were increased salivation, tremor, sedation, poor general condition. The poor general conditions were of slight intensity whereas the other symptoms were of moderate intensity.

Mortalities in males occurred from 1 hour after application until 24 hours after application. Females died 4 hours after application (see table 4.2-2).

Table 4.2-1: Acute oral toxicity

Dose [mg/kg bw]	Toxicological results*	Time of death	Mortality (%)
Males			
2000	4/5/5	1h - 24h	80
LD_{50} < 2000 mg/kg bw			
Females			
2000	3/5/5	4h	60
LD_{50} < 2000 mg/kg bw			

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

According to the results of this study the LD50 is considered to be below 2000 mg/kg bw for males and females.

Although the choice of dosing is not appropriate to determine an LD_{50}, the provided result, LD_{50} < 2000mg/kg, is reliable and can be considered as supportive information for classification (data on females).

Reference: Bomhard E. and Löser E. (1978 and 1992 (revised report)), Preventol CMK Untersuchung zur akuten oralen Toxizität an männlichen und weiblichen Wistar-Ratten Bayer AG, Institut für Toxikologie, Wuppertal, Germany Report No. 21862, 1992-11-24 (unpublished)

Guidelines: No, but in accordance with OECD 401 with deviations.
GLP standards: No, GLP was not compulsory during conduct of study.
Deviations: Yes

- only male rats were used
- acclimatisation period was only 3 days
- results of gross pathological examination were not reported

Study acceptable: Yes.
Test system: p-Chloro-m-cresol no further characterisation

Method:
10 male Wistar rats per group received 1000, 1500, 2000, 3100 and 5000 mg CMK/kg bw as a solution in Polyethyleneglycol 400 by single-dose oral gavage. In-life observations were made frequently on the day of treatment and twice each working day and once daily on weekends throughout the two-week observation period. Surviving animals were weighed before treatment, after one week and at study termination. Pathological-anatomical examinations were performed on all animals.

Results:
The clinical signs observed were increased diuresis, sedation, respiratory disturbance, side position, tremor and tonical cramps. Mortalities occurred from 1 hour after application until day 7 (see Table 4.2-3).

Table 4.2-2: Acute oral toxicity

Dose [mg/kg bw]	Toxicological results*	Time of death	Mortality (%)
1000	0/10/10	-	0
1500	4/10/10	2 d - 7 d	40
2000	7/10/10	3 h - 2 d	70
3100	8/10/10	3 h - 2 d	80
5000	10/10/10	1 h	100
LD_{50} = 1830 mg/kg bw			

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

The study has only been performed on males, which is not the recommended sex for testing in the current regulation. Thus, results could be considered of limited reliability. However, results of the other sex within the repeated exposure didn't reveal specific sensitivity between the two sexes. Therefore this study is considered acceptable.

Conclusion

The study results reveal that CMK is moderately toxic following acute oral exposure. The acute oral LD_{50} in the rat is 1830 mg/kg bw (males).

Reference: Miles Inc (1981), Acute oral toxicity of PCMC (p-chloro-m-cresol) to rats-Office of toxic substances Environmental Protection Agency 401 MStreet, SW Washington- Report No. 80-011-14 (unpublished)

Guidelines: no specify in the summary of registration dossier
GLP standards: no specify in the summary of registration dossier
Study acceptable: no
Test system: no specify in the summary of registration dossier

Method:

Adult male and female rats were used to test the acute oral toxicity of PCMC (p-chloro-m-cresol). Using Carbowax as the excipient, the test material was administered orally at graded dosages to groups of ten males and ten females. The rats were fasted for 19 hours prior to and at least one hour after dosing. The test material was administered at 2000, 2800, 3920, 5488 and 7683.2 mg/kg to male rats and at 1500, 2100, 2940, 4116 and 5762.4 mg/kg to female rats. The LD50 values were determined by probit analysis using an IBM 3.0 computer with the SAS Computer system (SAS Institute, Inc. Cary, Ne).

Results:

Symptoms of toxicity were observed in all treated rats. The incidence of mortality was dose-related. Rats that were found dead after treatment exhibited fluid in the stomach and/or intestines at gross necropsy. Rats that survived the l4-day observation period exhibited no gross lesions that could be related to treatment. The LD_{50} of PCMC was 5129 mg/kg (4432-6108 mg/kg) in male rats and 3636 mg/kg (3105-4370mg/kg) in female rats.
Available information is the summary of this study only and does not allow to conclude on the robustness of the advanced results. Indeed, the number of deaths by tested dose and by sex is not mentioned, which invalidates the results of this acute oral LD_{50} in the rat.

4.2.1.2 Acute toxicity: inhalation

Study for acute inhalation toxicity in the rat was conducted with the test substance p-chloro-m-cresol (CMK). This study is presented as supportive data for respiratory irritant endpoint.

Reference: Pauluhn, J (2003): PREVENTOL CMK Study on Acute Inhalation Toxicity Study in Rats according to OECD No. 403 Bayer AG, Toxicology, Wuppertal, Germany Report No. AT00251, 2003-01-28 (unpublished)

Guidelines: OECD-Guideline No. 403, US-EPA OPPTS 870.1300, 92/69/EEC Method B.2
GLP standards: Yes
Deviations: None
Study acceptable: Yes.
Test system: p-Chloro-m-cresol, Batch number: CHP 0117, purity: 99.8%

Method:
Five male and five female Wistar rats per dose group were nose-only exposed for four hours to a solid aerosol of CMK at target concentrations of 2000 and 3000 mg/m^3 air. The average dust concentrations reached were 1337 mg/m^3 and 2871 mg/m^3, which was the highest attainable aerosol concentration. 49% and 36.4% of the particles of the 2000 and 3000 mg/m^3 group were smaller than 3 µm in aerodynamic diameter, respectively. Analysis of the aerosol particle-size distribution from the breathing zone samples demonstrates that the aerosol generated was within the respirable range. Detailed clinical observations were made and body weights were taken during a two week post-exposure period. Rectal temperatures were determined within 30 minutes after exposure and reflex-measurements were performed on the first post-exposure day. A complete gross pathological examination was conducted on each rat at the end of the 2-week post-exposure period.

Results:
Exposure to the maximum technically attainable concentration of 2871 mg/m³ did not result in mortality. The clinical signs observed were indicative of respiratory distress, associated with subdued demeanour, decreased body weights, emaciation, and hypothermia:

- 1337 mg/m^3 males: abdomen bloated, salivation, cyanosis and tremor
- 1337 mg/m^3 females: nose: oedema/necrosis, giddiness, salivation
- 2871 mg/m^3 males: corneal opacity, nose: oedema/necrosis, cyanosis and tremor;
- 2871 mg/m^3 females: corneal opacity, nose: oedema/necrosis, emaciation, abdomen bloated, tremor.

In some rats the clinical signs lasted until the end of the 2-week post-exposure period. However, most rats showed evidence of recovery during the study period. Necropsy findings consisted of a less collapsed lung and secretions in the trachea. The hypothermia could be related to upper respiratory irritation caused by the high concentrations of aerosol tested.

Target Concentration [mg/m³ air]	Toxicological results*	Duration of clinical signs	Time of death	Mortality [%]
Males				
0	0/0/5	---	---	0
2000 Analytical concentrations (mean values): 1337 mg/m³	0/5/5	0d - 9d	---	0
3000 Analytical concentrations (mean values): 2871 mg/m³	0/5/5	0d - 12d	---	0
LC_{50} > 2871 mg/m³ air				
Females				
0	0/0/5	---	---	0
2000	0/5/5	0d - 8d	---	0
3000	0/5/5	0d - 14d	---	0
LC_{50} > 2871 mg/m³ air (= maximum practically attainable aerosol concentration)				

*first number = number of dead animals
second number = number of animals with signs (see above)
third number = number of animals used

Conclusion

According to the results, the test substance has an irritant potential to the respiratory tract, although it is of low acute inhalation toxicity to rats.

No mortalities occurred in acute studies by inhalation performed in rats at doses up to and including 2871 mg/m³.

4.2.1.3 Acute toxicity: dermal

Three studies for acute dermal toxicity in the rat were conducted with the test substance p-chloro-m-cresol (CMK).

Reference: Sturdivant DW (1999), Acute Dermal Toxicity Study with Preventol CMK Pastillen in Rats

Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, USA
Report No. 99-A22-FN, 1999-10-29 (unpublished)

Guidelines: US-EPA OPPTS 870.1200, OECD 402, JMAFF no. 4200
GLP standards: Yes
Deviations: Deviations from OECD 402:
The test was not performed as an LD50 test but similar to the oral ATC method (OECD 423).
Study acceptable: Yes.
Test system: p-Chloro-m-cresol, Batch number: E0136, purity: 99.9%

Method:
p-Chloro-m-cresol (CMK) was applied dermally to young-adult male and female Wistar Hanover rats. Six rats per sex and dose group received a single dermal dose of 0 and 5000 mg/kg bw test substance. 6 additional females received also a dose of 2000 mg/kg bw. The test substance was prepared as a paste using 0.5 mL (for the 2000 mg dose group) and 1 mL (for the 5000 mg dose group) of de-ionised water and applied occlusively to the shaved skin of the animals (~10% of body surface). The dressing was removed after 24 h and residual test substance was removed with paper towels moistened with tap water. The animals were inspected at least twice daily during the 14-day observation period (once on weekends and holidays) for mortality, moribundity and clinical signs of toxicity. The animals were weighed individually directly before administration (day 0) and for all surviving animals on day 7 and 14. Terminal body weights were recorded for all animals found dead. A complete gross necropsy was performed on all animals of the study.

Group mean body weight changes were evaluated with an Analysis of variance test and, where significant differences were detected, the Dunnett´s t-test was used to determine whether specific dose groups were significantly different from controls.

Results

Dose [mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death	Mortality (%)
Females				
0	0/5/6	day 0 - day 2	---	0
2000	2/6/6	day 0 - end	day 1, day 3	33.33
5000	3/6/6	day 0 - end	day 1	50
LD_{50} > 2000 mg/kg bw				
Males				
0	0/6/6	day 0 - day 1	---	0
5000	0/6/6	day 0 - end	---	0
LD_{50} > 5000 mg/kg bw				

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

As a result of dermal application of p-chloro-m-cresol, two females died in females treated with 2000 mg/kg bw and 3 females died in the 5000 mg/kg bw group. No mortalities occurred in male animals. Compound-related clinical signs were observed in all males and females of the treatment groups and included signs indicative of systemic toxicity as well as tissue damage and irritation at the application sites. A significant decrease in body weight gain was observed for the males of the 5000 mg/kg bw group on day 7 and was considered to be compound-related. Compound-related gross pathological observations were found at necropsy in all treated males and females.

Signs of systemic toxicity observed from the dose of 2000 mg/kg suggested a significant dermal absorption of CMK.

In another acute dermal administration study conducted on male and females rabbits (Rutter *et al*., 1979), the test substance was administered undiluted at dosages of 0 (control), 250, 500, 1000 and 5000 mg/kg, with 6 animal/sex/dose. The method used is similar to OECD 402 guideline.

All of the rabbits were observed immediately after dosing, at one and 4 hours, and twice daily thereafter for mortality and signs of toxic and pharmacologic effects for 14 consecutive days. Dermal responses were graded and scored on days 1, 3, 7, 10 and 14 according to the system of Draize (1959). Necropsies were performed at termination. Finally, the tissues were examined microscopically.

No death occurred during the 14 days period. Clinical observations consisted of clear jelly-like material on the anal region and in the pan in animals dosed at the 500 and 1000 mg/kg bw and marked anorexia noted in animals dosed at 250 and 5000 mg/kg bw. Fecal stains on the anal region and a swollen perineal region were noted in animals dosed at 250 mg/kg bw. Also, animals dosed at 5000 mg/kg exhibited slight depression, soft feces, and a red stain around the anal region.

Severe effects were observed at the site of contact. Indeed, the application of CMK resulted in marked epidermal and dermal necrosis for all animals of the treated groups at the end of the observation period. Moreover, obliteration of adnexal structures, marked pleocellular inflammatory infiltrate and acanthosis were noted. There was no appreciable difference in severity between the abraded and intact application sites or between rabbits receiving the various dosages of the test material.

The third study is an acute dermal toxicity study in wistar albino rat in the registration dossier.

The Wistar albino rats treated with the test compound did not show any clinical signs of intoxication throughout the period of observation. The LD_{50} value of chlorocresol in Wistar rats was found to be >2000 mg/kg body weight when applied dermally on the shaven back skin. The dermal LD_{50} value presented in registration dossier is consistent with those previously-mentioned.

Conclusion

CMK has a low order of acute toxicity in both male and female rats and rabbits exposed via the dermal route. The acute dermal LD_{50} in rat is higher than 2000 mg/kg (females and

males). The reasoning and the data that led TC C&L to classify are not found. However, based on the criteria of the CLP regulation as presented in table 3.1.1, CMK does not warrant any classification on the basis of its acute dermal toxicity.

4.2.1.4 Acute toxicity: other routes

No data

4.2.2 Human information

Type	Description	Results	reference
Poisoning incidents	Two male twin brothers were poisoned with Sagrotan containing milk, applied by their mother. Fatal outcome.	The deaths due to poisoning with Sagrotan could not be clearly ascribed to CMK contained in the disinfectant.	Joppich, 1960
Poisoning	11 cases of homicide using a CMK-containing disinfectant in a nursing home	Autopsies revealed bronchopneumonia and corrosive damage to the oesophagus/stomach.	Jonsson & Voigt, 1984
Poisoning incidents	3-year survey of poisoning cases with chlorocresol and cresol containing products	311 cases of acute poisoning, one with fatal outcome Poisoning symptoms: superficial skin burns, inflammation, burning sensation in mouth and throat, vomiting, abdominal pain, dysphagia, ulceration of oral mucosa, oedema of pharynx and glottis, salivation and coughing, haematemesis, dyspnoea, pneumonia, cough, collapse, phenolic staining of urine with liver and kidney damage	Wiseman et al., 1980
Poisoning incident	Treatment of accidental ingestion CMK containing disinfectant.	Patient survived. The observed toxicity (coma, convulsions, cyanosis) cannot be clearly ascribed to CMK.	Joppich, 1962

Several cases of poisoning after accidental or criminal oral application have been reported in the literature, some with fatal outcome (Joppich, 1960 and 1962; Wiseman et al., 1980; Jonsson & Voigt, 1984). The clinical signs which occurred after poisoning were, inter alia,

impaired general condition, vomiting, ulcerations of the oral mucosa, abdominal pain, salivation and coughing, dyspnoea, acidosis, tachypnoea and noisy breathing, and collapse. Evidence of liver and kidney damage were also noted in some cases.

However, it is difficult to conclude on the actual cause of death in these studies. Indeed, the exact amount of absorbed product is no known and fatal cases of poisoning cannot be clearly ascribed to CMK due to the more complex composition of disinfectants. Summary and discussion of acute toxicity

CMK is of low toxicity by the dermal route in rabbit.

According to the results, the test substance has an irritant potential to the respiratory tract, although it is of low acute systemic inhalation toxicity to rats.

CMK exhibits low to moderate toxicity by the oral route as a single dose, depending on the study. Results of acute oral studies showed LD_{50} higher than 2000 mg/kg bw in rats (males and females) and of 1830 mg/kg bw in male rats.

4.2.3 Comparison with criteria

The lowest acute toxicity estimate value is 1830 mg/kg bw (oral LD_{50} for male rats). This value lies within the range (300-2000 mg/kg) for classification as Acute Oral Tox. 4 (H302: Harmful if swallowed) under regulation (EC) 1272/2008.

No mortalities occurred in acute studies by inhalation performed in rats at doses up to and including 2871 mg/m^3 (= maximum practically attainable aerosol concentration) corresponding to 2.87 mg/L; therefore no classification is required.

The dermal LD_{50} lies above the classification cut-off of 2000 mg/kg bw under regulation (EC) 1272/2008; therefore no classification is required.

4.2.4 Conclusions on classification and labelling

Based on the results of the acute oral toxicity studies, CMK should be classified Acute Tox. 4 - H302 (Regulation (EC) 1272/2008).

No classification is proposed by inhalation and dermal route.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Route	Method Guideline	Species, Strain, Sex, No/group	Dosage, Duration of exposure	Value LD50/LC50	Reference
Inhalation	LC_{50} test OECD 403	Rat, Wistar ♂+♀, 5/sex/group	0, 1337, 2871 mg/m^3, 4 h	> 2871 mg/m^3	Pauluhn, 2003

4.3.1 Non-human information

Study for acute inhalation toxicity in the rat was conducted with the test substance p-chloro-m-cresol (CMK). This study is presented as supportive data for specific target organ toxicity and respiratory irritant endpoint.

Reference: Pauluhn, J (2003): PREVENTOL CMK Study on Acute Inhalation Toxicity Study in Rats according to OECD No. 403 Bayer AG, Toxicology, Wuppertal, Germany Report No. AT00251, 2003-01-28 (unpublished)

Guidelines: OECD-Guideline No. 403, US-EPA OPPTS 870.1300, 92/69/EEC Method B.2
GLP standards: Yes
Deviations: None
Study acceptable: Yes.
Test system: p-Chloro-m-cresol, Batch number: CHP 0117, purity: 99.8%

Method:
Five male and five female Wistar rats per dose group were nose-only exposed for four hours to a solid aerosol of CMK at target concentrations of 2000 and 3000 mg/m^3 air. The average dust concentrations reached were 1337 mg/m^3 and 2871 mg/m^3, which was the highest attainable aerosol concentration. 49% and 36.4% of the particles of the 2000 and 3000 mg/m^3 group were smaller than 3 µm in aerodynamic diameter, respectively. Analysis of the aerosol particle-size distribution from the breathing zone samples demonstrates that the aerosol generated was within the respirable range. Detailed clinical observations were made and body weights were taken during a two week post-exposure period. Rectal temperatures were determined within 30 minutes after exposure and reflex-measurements were performed on the first post-exposure day. A complete gross pathological examination was conducted on each rat at the end of the 2-week post-exposure period.

Results:
Exposure to the maximum technically attainable concentration of 2871 mg/m³ did not result in mortality. The clinical signs observed were indicative of respiratory distress, associated with subdued demeanour, decreased body weights, emaciation, and hypothermia:

- 1337 mg/m^3 males: abdomen bloated, salivation, cyanosis and tremor
- 1337 mg/m^3 females: nose: oedema/necrosis, giddiness, salivation
- 2871 mg/m^3 males: corneal opacity, nose: oedema/necrosis, cyanosis and tremor;
- 2871 mg/m^3 females: corneal opacity, nose: oedema/necrosis, emaciation, abdomen bloated, tremor.

In some rats the clinical signs lasted until the end of the 2-week post-exposure period. However, most rats showed evidence of recovery during the study period. Necropsy findings consisted of a less collapsed lung and secretions in the trachea. The hypothermia could be related to upper respiratory irritation caused by the high concentrations of aerosol tested.

Target Concentration [mg/m³ air]	Toxicological results*	Duration of clinical signs	Time of death	Mortality [%]
Males				
0	0/0/5	---	---	0
2000 Analytical concentrations (mean values): 1337 mg/m³	0/5/5	0d - 9d	---	0
3000 Analytical concentrations (mean values): 2871 mg/m³	0/5/5	0d - 12d	---	0
LC_{50} > 2871 mg/m³ air				
Females				
0	0/0/5	---	---	0
2000	0/5/5	0d - 8d	---	0
3000	0/5/5	0d - 14d	---	0
LC_{50} > 2871 mg/m³ air (= maximum practically attainable aerosol concentration)				

*first number = number of dead animals
second number = number of animals with signs (see above)
third number = number of animals used

Conclusion

According to the results, the test substance has an irritant potential to the respiratory tract, although it is of low acute inhalation toxicity to rats.

No mortalities occurred in acute studies by inhalation performed in rats at doses up to and including 2871 mg/m^3.

4.3.2 Human information

No data.

4.3.3 Summary and discussion of Specific target organ toxicity – single exposure

Oedema and necrosis located in nose, reddened nostrils with red encrustations, nasal discharge, and abundant secretions in the trachea were signs of an upper respiratory irritation. Based on these results, CMK is considered an irritating to respiratory system.

4.3.4 Comparison with criteria

The criteria for STOT SE 3 for respiratory irritation states that "this evaluation is primarily based on human data" but that "useful information may be obtained from the single and repeated inhalation toxicity tests" in animals. Effects that are relevant to consider for respiratory irritation according to the CLP criteria are clinical signs such as dyspnea and rhinitis, and histopathology findings such as hyperaemia, oedema, minimal inflammation, thickened mucous layer, which are reversible effects.

In the absence of relevant human data for CMK, the acute inhalation study provides some relevant evidence of respiratory irritation. The clinical signs observed were indicative of respiratory distress, associated with subdued demeanour, decreased body weights, emaciation, and hypothermia. In some rats the clinical signs lasted until the end of the 2-week post-exposure period. However, most rats showed evidence of recovery during the study period. Necropsy findings consisted of a less collapsed lung and secretions in the trachea. Therefore the evidence of respiratory irritation comes only from the observed clinical signs.

As the tested substance is a white solid, it cannot be excluded that the mechanical effect of solid particles contributed to the irritation observed at high concentrations. However, clinical signs indicating respiratory irritation are not only observed at the high dose of 2.87 mg/L, but also at 1.33 mg/L, and therefore mechanical irritation may not fully explain the observed clinical signs.

4.3.5 Conclusions on classification and labelling

Based on the observed irritation of respiratory system, a classification STOT SE 3 H335: may cause respiratory irritation, according to Regulation EC No 1272/2008 is proposed for CMK.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Species	Method	Average score 24, 48h		Reversibility	Result	Reference
		Erythema	Oedema			
Rabbit	Dermal NZW rabbits US method (21 CFR 191.11≅ OECD 404) Non-GLP	1.9*	0.5*	not reported	Irritating	Lamb, 1976

* Average scores at 24 and 48 hours for all 6 animals.

4.4.1.1 Non-human information

The potential of CMK to irritate skin was tested in New Zealand White albino rabbits (Lamb, 1976).

Reference: Lamb, D.W. (1976): Preventol CMK – The eye and dermal irritancy of Mobay sample p-Chloro-m-cresol. Chemagro Agricultural Division, Mobay Chemical Corp. R&D Unpublished report N° 50874 - Date: 1976-11-30

Guidelines: Federal Hazardous Substances Act, 21 CFR 191.11 (≅ OECD 404)
GLP standards: No, study was conducted before the enactment of GLP regulations
Deviations: Deviations from OECD 404:

- Test material was not characterized
- Sex and age of the test animals were not reported
- Test included application on abraded skin
- First scoring at 4 hours instead of at 60 minutes.
- No reading was performed at 72 h post exposure

Study acceptable: Yes.
Test system: p-Chloro-m-cresol, no further characterisation

Method:
The procedure followed the current OECD Guideline 404 with minor deviations. The test was conducted by applying 500 mg CMK to the shaved skin of rabbits for 4 h. The test sites were shaved. The application sites were loosely covered with 5 × 5 cm² gauze patches recovered by plastic. The Draize scale was used for scoring the skin reactions at 4, 24, 48, and 96 h post exposure.

FR-MSCA considers that the duration of the observation period (96h) is not sufficient to evaluate the reversibility of the effects observed.

Results
The scores are reported in the table below:

OBSERVATION TIME	RABBIT NO.											
	53		54		57		58		63		64	
	E*	O*	E	O	E	O	E	O	E	O	E	O
4 H	0	4	0	3	0	1	0	1	0	1	0	2
24 H	2	2	2	1	2	1	2	0	2	0	2	1
48 H	2	1	2	0	2	0	2	0	2	0	1	0
MEAN VALUE 24 +48 H	2.0	1.5	2.0	0.5	2.0	0.5	2.0	0.0	2.0	0	1.5	0.5
MEAN VALUE 24 +48 H, ALL ANIMALS	1.9	0.5										

* E: erythema, O: oedema (according to Draize Score)

Although the average dermal irritation score is equivalent to 1.9 for erythema – which is at the limit of classification but do not lead to classification, FR-MSCA is concerned by the following points.

- the lack of data relative to reversibility. Indeed the effects (erythema) are still present at 96h and their reversibility cannot be determined.

- severe and increasing effects at the site of contact have been observed in an acute dermal administration study conducted on male and females rabbits (Rutter *et al*., 1979). In this study, the application of CMK resulted in marked epidermal and dermal necrosis for all dosages and all animals at the end of the observation period.

Nevertheless, no data is available on reversibility of the observed effects. The erythema is still present at 96h (last examination time). In consequence, no reversibility was observed at the end of the study.

It should be noted that a study performed on one rabbit present in registration dossier shows results consistent with those previously-mentioned.

4.4.1.2 Human information

See part 4.6.1.2, in particular, there is one case of irritation reported in Geier et al., 1996

4.4.1.3 Summary and discussion of skin irritation

Under the conditions of the study, local inflammatory reaction was observed after topical cutaneous application of CMK to the skin of rabbits for 4 hours loosely covered. CMK was designated irritating to the skin supported by the results observed in the dermal acute study (paragraph 4.2.1.3).

4.4.1.4 Comparison with criteria

Based on the Lamb‘s study, the average dermal irritation score, after duration of exposure of 4 hours, is equivalent to 1.9 for erythema, which is below 2.3, which is the classification threshold according to the 1272/2008 regulation.

Nevertheless, no data is available on reversibility of the observed effects. The erythema is still present at 96h (last examination time). In consequence, no reversibility was observed at the end of the study. Therefore, it can be considered that inflammation persists to the end of the observation period. Normally, this should be seen in at least 2 animals after 14 days, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling. In absence of details, this finding is considered to be relevant for classification as Skin irritant cat. 2.

Finally, local effects are also observed in the acute dermal study supporting the classification proposal.

4.4.1.5 Conclusions on classification and labelling

In conclusion, a classification Skin irrit. 2; H315 : Causes skin irritation according to Regulation EC No 1272/2008 is proposed for CMK.

4.4.2 Eye irritation

An eye irritation study with CMK was performed in New Zealand White albino rabbits (Lamb, 1976) and does not modify the current classification. Therefore it is not presented in this dossier.

4.4.3 Respiratory tract irritation

For this end point, please refer to Part 4.3 Specific target organ toxicity-single exposure (STOT SE)

Route	Method Guideline	Species, Strain, Sex, No/group	Dosage, Duration of exposure	Value LD50/LC50	Reference
Inhalation	LC_{50} test OECD 403	Rat, Wistar ♂+♀, 5/sex/group	0, 1337, 2871 mg/m³, 4 h	> 2871 mg/m³	Pauluhn, 2003

According to the results, the test substance has an irritant potential to the respiratory tract.

Oedema and necrosis located in nose, reddened nostrils with red encrustations, nasal discharge, and abundant secretions in the trachea were signs of an upper respiratory irritation. Based on these results, CMK is considered as an irritating to respiratory system**.**

No human data is available.

4.4.3.1 Conclusions on classification and labelling

Based on the observed irritation of respiratory system, a classification STOT SE 3 H335: may cause respiratory irritation, according to Regulation EC No 1272/2008 is proposed for CMK.

4.5 Corrosivity

A classification Skin irrit. 2; H315: Causes skin irritation according to Regulation EC No 1272/2008 is proposed for CMK. There is no additional data for corrosivity.

(S*ee section 4.1).*

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

Species	Method	Concentrations	Number of animals sensitised/ total number of animals	Result	Reference
Mouse	≅ OECD 429 modified LLNA (IMDS)	0, 1, 10 and 50%	Cell count indices: 1.0, 0.99, 0.71 and 1.28 for the dose groups, respectively (>1.25, positive level)	Weakly sensitising	Vohr, 2000
Guinea pig	≅ OECD 406 MAGNUSSON & KLIGMAN-Test	Intradermal induction: 25% (males) and 1% (females), Topical induction: 25% (males) and 1% (females) Challenge: 25% and 50% (females), 25% and 12,5% (males)	Induction/challenge: 25%/25%: 13/15 (males) Induction/challenge: 1%/50%: 4/15 (females)	strongly sensitising weakly sensitising	Bomhard, 1980

4.6.1.1 Non-human information

The skin sensitising potential of CMK was tested in two different studies.

Reference: Vohr HW (2000): Preventol CMK, Pastillen LOCAL LYMPH NODE ASSAY IN MICE(LLNA/IMDS) Bayer AG, Department of Toxicology, Wuppertal, Germany Report No.: PH 30408, 2000-11-13 (unpublished)

Guidelines: LLNA test - OECD Guideline No. 429
US-EPA OPPTS 870.2600
EEC Method B.6
No statement claimed, but also in accordance with EEC Method B.42 (= OECD 429) with modifications.
GLP standards: Yes.
Deviations:

- Measuring of cell counts instead of radioactive labelling
- Additional ear swelling measurement
- Sacrifice 1 day after last treatment

Study acceptable: Yes. This study was presented only because of the positive result, no validated protocol is available for this modified LLNA.

Test system: p-Chloro-m-cresol, Batch number: E 0136, purity: 99.9%

Method:
A modified local lymph node assay (LLNA) was performed in NMRI mice to determine the sensitising potential and the irritating potential of CMK. Instead of radioactive labelling cell counts were measured. Therefore animals were sacrificed one day after the last application instead of three days after the last application. In addition, a measurement of the ear swelling after treatment was included in the test.

Groups of 6 female NMRI mice received 25 µL of test item formulations applied epicutaneous onto the dorsal part of both ears on three consecutive days. The formulations contained 0, 1, 10 and 50% test item in DEA 133. After the third application animals were sacrificed and the auricular lymph nodes were transferred into sterile physiological saline. After preparation the weight and cell counts were determined and the stimulation index was calculated by dividing the absolute number of weight or cell counts of the substance treated lymph nodes by the vehicle treated ones.

In addition, on day 0 and 3 the ear swelling was measured using a spring-loaded micrometer and the mean ear swelling was determined.

Treatment	LLNA/IMDS	Observations/Remarks
	Day of treatment or time point	
Application 1	Day 0	25 µL / ear
Application 2	Day 1	25 µL / ear
Application 3	Day 2	25 µL / ear
Draining lymph node preparation	Day 5	Modification of LLNA test; no radioactive labelling
Measuring of cell counts	Day 5	Measuring of cell counts instead of scintillation counting

After sacrifice the ear weights were determined and the ratio ear swelling/ear weight was calculated. For the determination, an 8 mm in diameter ear punch was weighed.

Results:
The stimulation index is calculated by dividing the absolute number of weight or cell counts of the substance treated lymph nodes by the vehicle treated ones (see table below).

Result of skin sensitisation test (LLNA/IMDS)

Direct LLNA / number of animals in group		
Dose (%)	**Weight index**	**Cell count index**
	mean ± SD in %	
0	1.00 ± 17.78	1.00 ± 30.72
1	1.07 ± 18.06	0.99 ± 35.59
10	0.97 ± 19.06	0.71 ± 33.61
50	1.34 ± 20.43	1.28 ± 27.04

Result of skin sensitisation test (ear swelling)

Ear swelling (NMRI mice, female, 6 animals/group, in 0.01 mm)			
Dose (%)	**day 0**	**day3**	**Index day 3**
	mean ± SD in %		
0	19.75 ± 5.34	19.92 ± 8.14	1.00
1	19.83 ± 4.73	19.50 ± 6.74	0.98
10	19.58 ± 7.04	19.50 ± 6.38	0.98
50	19.17 ± 6.61	19.33 ± 6.74	0.97

Result of skin sensitisation test (ear weight)

Ear weight (NMRI mice, female, 6 animals/group, in mg per 8 mm diameter punch)		
Dose (%)	**day 3 (mean ± SD in%)**	**Index day 3**
0	14.49 ± 7.27	1.00
1	13.60 ± 6.39	0.94
10	13.84 ± 8.11	0.96
50	13.48 ± 5.17	0.93

The NMRI mice showed a slight increase in the stimulation indices for cell counts and for weights of the draining lymph nodes after application of the test item.

The results showed that the test item (Preventol CMK, Pastillen) has a weak sensitising potential in mice after dermal application. There was an increase compared to vehicle treated animals regarding the cell counts and the weight of the draining lymph nodes in the highest dose group.

An irritating potential at the same doses measured by ear swelling or ear weights could not be determined.

It should be noted that this modified LLNA was presented only because of the positive result, no harmonised criteria of classification exists for this test.

Reference: Bomhard, E. & Löser, E. (1980): Preventol CMK–Investigation of sensitizing effect (Maximisation test after Magnusson and Kligman)
Bayer AG, Institute of Toxicology, Wuppertal, Germany
Report No.: 8897, 1980-01-23 (unpublished)

Guidelines: No. Study was conducted prior to establishing of accepted guidelines, but general accordance with EC Method B.6 (= OECD 406) can be stated.
GLP standards: No, study was conducted prior to the enactment of GLP principles.
Deviations: Deviations from OECD 406:

- No reliability check was included (no positive control).
- The concentration used for induction was not irritating.

Study acceptable: Yes**.**
Test system: p-Chloro-m-cresol, Batch number: 54 603 00, purity: 100%

Method:
Groups of 15 Pirbright White guinea pigs each were used in two legs of the study. In the 1st study, male animals received intradermal and topical induction treatments with 25% CMK in vehicle. In the 2nd study, female animals were induced with a 1% solution of CMK. Both studies included respective vehicle control groups (Lutrol 300/ethanol (3:1)) with 15 animals each. Each induction exposure was 48 h in duration.

In the 1st study, guinea pigs were challenged with a 25% (right flank) or 12.5% (left flank) CMK suspension in vehicle.

In the 2nd study, guinea pigs were challenged with a 50% (right flank) or 25% (left flank) CMK suspension in vehicle

The challenge was performed two weeks after the last induction application. Fifteen naïve animals each also received an identical challenge application with CMK. Each challenge exposure lasted 6 h. The Magnusson-Kligman scoring system was used to rate the skin reactions.

Results:

24 h after challenge	1st study		2nd study	
	CMK	vehicle	CMK	vehicle
	1/7/15*	0/0/15	0/4/15	0/0/15
48 h after challenge	1st study		2nd study	
	CMK	vehicle	CMK	vehicle
	3/13/15	0/1/15	0/2/15	0/0/15

*(left flank reactions/right flank reactions/number of animals)

Concentrations of the test substance used at injection and topical application for induction should have been the highest to cause mild to moderate skin irritation (see mentioned deviations). This is not the case for the topical application, and this is not determined for the intradermal injection. Besides, this could explain the weak results of the second study where the 1% concentration used at day 0 is probably too low to develop an appropriate immune response.

In the 1st study, skin reactions were noted in 13 out of 15 animals, CMK is thus considered a skin sensitizer.

4.6.1.2 Human information

Type	description	Results	Reference
Allergy report	40-year-old man with allergic contact dermatitis to chlorocresol used in several topical corticosteroid preparations.	Patch test revealed a positive reaction to CMK (Previous testing showed a positive reaction to chromium).	Gomez *et al*., 2013
Allergy report	Daily treatment of dermatitis with diluted betamethasone cream. After treatment was stopped the dermatitis cleared.	Patch test revealed a positive reaction to CMK. (Previous testing showed a positive reaction to chloroxylenol)	Burry *et al*., 1975
Allergy report	Patch tests with a standard series + CMK (1% in pet) in consecutive eczema patients	None out of 671 patients showed a positive reaction.	Andersen & Veien, 1985
Allergy report	Patch test on dermatitis patients in two regions of	Region 1: 0.8% of 651 patients had a positive	Wilkinson *et al*., 1980

	England Occlusive exposure for 2 days	reaction Region 2: 0.7% of 1029 patients reacted positive to CMK	
Epidemiological study on metal workers in the Netherlands	286 metal workers exposed to MWF were tested	Patch tests with standard series and components of MWFs. CMK produced no positive reaction.	de Boer *et al*., 1989
Allergy report	Patch test result of the North American Contact Dermatitis Group	None of 220 tested persons showed a positive response to CMK	Rudner, 1977
Allergy report	Patch tests in metal workers	Of 277 patients tested, only one showed a questionable result, no positive reactions were noted.	Uter *et al*., 1993
Allergy report	Patch tests with several substances	1 out of 127 patch tested patients showed a positive reaction to CMK	Angelini *et al*., 1975
Retrospective study	Patch test reactions to a preliminary preservative series	2044 patients patch tested with CMK (764♂, 1280♀) 5 patients (0.24%) showed a positive reaction, 9 (0.44%) had a questionable result, and 2 (0.1%) had an irritating reaction	Brasch *et al*., 1993
Epidemiological study (analysis of patch test data)	Patch tests with antiseptics and industrial chemicals	1132 patients patch tested with CMK 2 patients (0.2%) showed a positive reaction, 4 had a questionable result, and 1 had an irritating reaction	Geier *et al*., 1996
Allergy report	Two cases of allergy to chlorocresol and propylene glycol in a steroid cream (patch tests were performed)	Skin sensitisation to chlorocresol and propylenglycol. In the first case a chloroxylenol containing paste was the primary sensitiser for chlorocresol. In the second case, the steroid component of the cream masked the skin reaction for at least 48 hours.	Oleffe *et al*., 1979
Allergy report	One case of allergy to CMK after irritant dermatitis from tri-butyl tin oxide	Skin sensitisation to CMK contained in a cream used for treatment of an irritant contact dermatitis.	Lewis & Emmett, 1987
Allergy report	Report of immediate and delayed sensitivity to chlorocresol Acute dermatitis of face and feet after contact to	Patch tests reveal positive response to CMK	Goncalo *et al.*, 1987

	disinfectant. Treatment with steroid cream, containing CMK, did not resolve the dermatitis.		
Allergy report	Contact urticaria after use of disinfectants for chicken incubators.	Patch tests reveal sensitivity to CMK (10%)	Freitas & Brandao, 1986
Allergy report	Contact allergy to chlorocresol contained in handling glue and in corticosteroid cream used for treatment of the allergy	Patch tests reveal sensitivity to CMK.	Dooms-Goossen *et al.*, 1981
Allergy report	Treatment of an eczema with cream containing CMK caused worsening	Chlorocresol sensitivity induced by treatment with steroid creams, used for treatment of allergic contact dermatitis.	Archer & McDonald, 1984

There are no medical surveillance data of manufacturing plant personal available. Superficial skin burns were also noted after skin contact with spilt CMK containing liquids. Several epidemiological studies on patients with dermatitis or dermatoses have been performed. Patch tests were performed on a large number of patients. The percentage of positive responses was less than 0.5%, the percentage of questionable results was only slightly higher. Nevertheless, some cases of skin sensitisations due to CMK containing steroid creams, used for treatment of dermatoses, or occupational used disinfectants or other substances were observed (Gomez *et al* 2013, Oleffe *et al*., 1979; Lewis & Emmett, 1987; Freitas & Brandao, 1986; Dooms-Goossen *et al*., 1981 and Archer & McDonald, 1984). Hypersensitivity reactions after intravenous and sub-cutaneous applications of CMK-preserved heparin were also noted (Hancock & Naysmith, 1975).

4.6.1.3 Summary and discussion of skin sensitisation

The technical grade CMK showed in both Magnusson-Kligman test and modified LLNA (counting cell method) a slight to strong sensitising effect. The modified LLNA (Integrated Model for the Differentiation of Skin reactions = IMDS) was done with the following modifications: instead of radioactive labelling cell counts were measured. Therefore animals were sacrificed one day after the last application instead of three days after the last application. In addition, a measurement of the ear swelling after treatment was included in the test. However, this protocol is not validated yet.

Moreover, the publication of Basketter et al. "An evaluation of performance standards and non-radioactive endpoints for the LLNA" (2008) reporting the conclusions of the ECVAM workshop 65, mention that the method of counting cell corresponds to a major variation from the initial LLNA.

Consequently, this modified LLNA was presented only because of the positive result, no harmonised criteria of classification exists for this test.

Therefore, considering the results of the animal studies together with the human data, CMK is considered to be a skin sensitiser.

4.6.1.4 Comparison with criteria

Based to the results in the maximization assay and considering the classification criteria in the CLP regulation, the test compound fulfils the criteria to be classified as Skin Sens. Category 1B (H317) since the intradermal induction concentration was 25% higher than the threshold of 1% and the sensitisation rate was 47% at 24 hours and 87% at 48 hours after challenge, also higher than the threshold of 30% of sensitised animals.

This proposed classification is supported by a slight increase in the stimulation index observed in the modified LLNA test.

4.6.1.5 Conclusions on classification and labelling

Based on the positive Magnusson-Kligman test and LLNA, CMK should be classified as Skin Sens. 1B H317: May cause an allergic skin reaction according to Regulation EC No 1272/2008.

4.6.2 Respiratory sensitisation

No data and no indications are available for this endpoint.

4.7 Repeated dose toxicity

Not considered in this dossier

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not considered in this dossier

4.9 Germ cell mutagenicity (Mutagenicity)

Not considered in this dossier

4.10 Carcinogenicity

Not considered in this dossier

4.11 Toxicity for reproduction

Not considered in this dossier

4.11.1 Effects on fertility

Not considered in this dossier

4.12 Other effects

Not considered in this dossier

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference

BIOTIC DEGRADATION			
Ready biodegradation 84/449 EEC Appendix V C.6: /OECD 301 D	Day 15 - 78%	Initial TS concentration:4.5 mg/L Inoculum is a population of activated sewage sludge microorganisms	Müller, 1992, amended by Weyers in 2007 and Neuhahn in 2012
Ready biodegradation Draft OECD. Doc. No. CDUP/ 89.104/13.6	Day 28 - 4% Day 56 - 32%-52%	Initial TS concentration:1.71 mg/L Inoculum is a population of activated sewage sludge microorganisms.	Hanstveit and Pullens, 1993
Inherent Biodegradation OECD 302A and 301D	Day 28 – 0% (16 days adaptation) Day 28 – 78% (35 days adaptation)	Initial TS concentration:5.0 mg/L Inoculum is a population of domestic sludge microorganisms.	Thompson, 1993
Simulation test : Degradation in Two Water/Sediment Systems OECD 308	**DT50 (days)** **1 Pond** Water 1.74 (20°C) 3.30 (12°C)* Total system 1.90 (20°C) 3.60 (12°C)* **2 River** Water 1.07 (20°C) 2.03 (12°C)* Total system 1.22 (20°C) 2.31 (12°C)* **DT90 (days)** **1 Pond** Water 5.78 Total system 6.31 **2 River** Water 3.57 Total system 4.05	Initial TS concentration : 200 µg/L	Möndel, 2009
Aerobic degradation in soil No specific guideline (literature data) 40 days incubation at 25°C in the dark, at field capacity moisture	**DT50 (days)** **Acid silty clay and neutral sandy clay** 21 (59.4 at 12°C*)	Initial TS concentration : 0, 10, 1000 mg/kg	Sattar, 1989
Aerobic degradation in soil No specific guideline (literature data) 64 days incubation at 20°C in the dark, at 80% field capacity	**DT50 (days)** **1 Acidic sandy loam** 1.4 (2.7 at 12°C*) **2 Basic sandy silt loam** 4.2 (8.0 at 12°C*)	Initial TS concentration : 10 and 20 mg/kg	Loehr and Matthews, 1992
Aerobic degradation in soil No specific guideline (literature data) 19 days incubation at 22-23°C in	**DT50 (days)** **1 Sandy silt loam** 5.4 (12.5 at 12°C*)	Initial TS concentration : 10 mg/kg	Nitsche, 2011

the dark, 80% field capacity			
ABIOTIC DEGRADATION			
Hydrolysis EC Guideline 92/69/EC	DT50 at 50°C pH 4, 7, 9 : Not relevant due to the hydrolytic stability of the compound	Stable	Erstling and Feldhues, 2001 a
Photolysis in water OECD Guidance Document on direct phototransformation of chemicals in water (1997)	Not relevant due absorbance properties of the compound	Stable	-

* recalculated value to reflect an average EU outdoor temperature

5.1.1 Stability

The hydrolysis of CMK was studied in aqueous buffered solutions at pH 4 (citric acid/potassium hydroxide/sodium chloride buffer), pH 7 (potassium dihydrogen phosphate/di-sodium hydrogen phosphate buffer) and pH 9 (borax/hydrochloric acid buffer) according to EC Guideline 92/69/EC, C.7 (Erstling and Feldhues, 2001 a). Buffer solutions spiked with the test substance were kept at 50°C for 5 days. The measurement of CMK was conducted via HPLC-UV. CMK was stable under acidic (pH 4), neutral (pH 7) and alkaline (pH 9) conditions at 50°C. The test substance still accounted for about 100% in the solutions at termination of the experiments compared to initially applied amounts. The formation of hydrolysis products was not followed in the course of the study. However, due to the hydrolytic stability of the test substance this aspect is of no relevance.

The photodegradation of CMK was determined according to ECETOC technical report No. 12 (1984) (Wilmes, 1988) but the study was not considered acceptable by RMS due to numerous deficiencies as the absence of irradiation apparatus description.

Nevertheless, p-chloro-m-cresol was identified by UV/VIS spectrum with a maxima at 228 nm (ε = 9625 l mol-1cm-1) and 281 nm (ε = 2241 l mol-1cm-1). No UV absorbance was noted above 290 nm. And according to OECD Guidance Document on direct phototransformation of chemicals in water (1997) *"*No direct photoreaction is possible without absorption of light quanta. Only quanta of UV/visible light are energetic enough to break bonds between atoms in a molecule and only the wavelength range 290-800 nm is relevant for photolysis in the water compartment. As a consequence, chemicals that absorb light significantly only in the UV region below 290 nm and in the infra-red above 800 nm cannot undergo direct photolysis in the water compartment.*"* As the UV absorbance of the molecule is below the solar irradiation at the earth's surface, direct photodegradation is unlikely under environmental conditions.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Several tests regarding the ready biodegradability of CMK have been conducted.

A first test (closed bottle test - OECD guideline 301 D) was conducted in 1992 (Müller, amended by Weyers in 2007 and Neuhahn in 2012). The test substance (4.5 mg/L) was incubated with

activated sludge for a period of 28 days at 20 to 21 C. The inoculum came from a laboratory scale unit receiving exclusively domestic sewage from the Wupper area water authority (Germany). CMK degraded by 4 % (BOD/ThOD) within the first 5 days. After 15 days, 78 % biodegradation was reached. At the end of the 10-day window, the difference of extremes of replicate values of the removal of the test chemical is 39% when expressed as oxygen content. However, when the percentage of degradation of the substance is taken into account, the difference of extremes of replicate value is 12%. Based on this last parameter, the validity criterion dealing with the difference between the replicates is considered as fulfilled. Validity criteria are therefore fulfilled and, CMK could be considered to be ready biodegradable.

A further test on ready biodegradability was conducted according to Draft OECD guideline, Doc. No. CDUP/89.104/13.6 (Hanstveit and Pullens, 1993), which is in general accordance with OECD 301 D (closed bottle test). Activated sludge inoculum of two concentrations, i.e., 2.5 mL and 7.6 mL inoculum/L was treated with CMK at a concentration of 1.71 mg/L and incubated for 56 days. The biodegradation (BOD/COD) of CMK started between day 28 and 42 days of incubation. After 56 days, a biodegradation of 32% was found for the low inoculum activity (2.5 mL/L) and 52% for the high inoculum activity (7.6 mL/L). Hence, after an approximately four week adaptation period a significant degradation of the test compound could be noticed. It was postulated that CMK might have inhibited the endogenous activity of the microorganisms during the first weeks. However, based on the validity criteria of OECD guideline 301 (criteria for inhibitory substances: less than 25 % degradation in the toxicity control) CMK cannot be denoted as such. It is important to note that for the inoculum activity of 7.6 mL/L, oxygen depletion in the inoculum blank exceed 1.5 mg dissolved oxygen/L after 28 days and the test is not considered valid for this inoculum activity.

Additional studies have been submitted by the applicant as supportive data.

Neuhahn (1981) summarized the results of ready biodegradability tests with CMK (according to OECD guideline 301 D, closed bottle test), which had been conducted in the years 1976, 1979 and 1981 . Activated sludge was treated with the test substance at a concentration of 2 to 10 mg/L and incubated for 28 days at 28°C. As test parameter the biochemical oxygen demand (BOD) as a percentage of the chemical oxygen demand (COD) was calculated. Whereas the test conducted in 1976 revealed CMK to be ready biodegradable (70% biodegradation after 10 days), the two later experiments came to the conclusion of 43% (after 20 days) and 52% (after 28 days) biodegradation. The principal deficiency of this study is the lack of information on the inoculums origin; in fact the use of an adapted inoculum cannot be excluded.

A test conducted according to OECD guideline 301 C (MITI test) came to the result of CMK being ready biodegradable (N.N., 1985). Activated sludge was spiked with the test compound at a concentration of 100 mg/L and incubated for 28 days. Besides the BOD (oxygen depletion) the chloride content was determined at the start and end of the test. Already after 6 days a biodegradation (BOD/ThOD) of 61.8 % could be observed. CMK was completely mineralised (> 90%) after 16 days. This result was confirmed by the release of chloride. Nevertheless, the methodology and data reporting are not sufficient to state that there was no adaptation of the inoculum before the test and to check the validity criteria because of the lack of the raw data.

Based on all these results and mainly on the key study by Müller (1992), CMK is considered as ready biodegradable.

The inherent biodegradability of CMK was determined according to OECD guidelines 302 A and 301 D (Thompson, 1993). 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 28-days closed bottle tests and the BOD was measured following a single application of the test substance at a concentration of 5 mg/L.

Within 9 days of adaptation in the SCAS unit, approximately 95% removal of the test substance occurred. After 35 days of SCAS acclimatisation, the effluent concentration was below the detection limit of the analytical method (>97 % removal) and this was maintained up to day 58. Some degree of sorption to sludge solids was observed in the course of the first 14 days of incubation.

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 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 already after 5 days. At termination of the test (after 28 days) 78 % biodegradation (BOD in % of ThOD) was achieved. Chemical analysis conducted on days 5 and 20 showed >98 % removal of the test substance. The lack of biodegradation in the first closed bottle test suggests that, at this stage, the micro-organisms capable to degrade CMK in the SCAS system 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 was able to exhaustively biodegrade CMK.

The supportive study Neuhahn (1981) summarized the results of inherent biodegradability tests with CMK, which had been conducted in the years 1979 and 1981. An aqueous solution of CMK was kept in contact with a bacterial suspension and gassed with air over a period of 8 – 14 days. After this adaptation period the bacteria were used in a closed bottle test according to OECD 301 D. Both tests revealed a high degree of biodegradation, i.e. 89% (BOD in % of COD) at day 20 for the test of 1979 and 62% at day 28 for the experiment of 1981.

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**.

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems

A study conducted according to OECD Guideline 308 was submitted for water/sediment degradation (Möndel, 2009) and was considered as the key study. Water/sediment distribution and degradation were studied in two natural systems (‘Pond’ and ‘River’) with ^{14}C-labeled chlorocresol (200 µg/L) incubated in the dark at 20 ± 2°C for up to 35 days. It was concluded that chlorocresol dissipated rapidly with a DT_{50} in the water of 1.07 to 1.74 days, and a DT_{50} of 1.22 to 1.90 days in the whole system, at a reference temperature of 20°C. The half life has been recalculated at 12°C and the values are presented in the **Erreur ! Source du renvoi introuvable.**.

Concerning the extractable residues of CMK in sediment, it reaches a peak at day 3 for Pond with 3.82% AR and at day 7 for River with 14.71 % AR and slightly decreases until day 35 to reach 1.95% in Pond system and 1.56% in River system. Non-extractable residues are high from the first day after application (22.6% AR for Pond system and 15.5% AR for River system) and increased continuously during the incubation period to reach maximum portions of 54.2% AR (Pond) 28 days and 54.3% AR (River) 14 days after application. Thereafter the amount of non-extractable residues decreases to 46.4% AR (Pond) and 52.4% AR (River) on the last sampling day.

The amount of mineralisation product $^{14}CO_2$ increased continuously and amounted to 23.9% AR (Pond) and 37.0% AR (River) at 35 days.

After reaching maximum values 3-4 days after application (26.98% AR in water phase at day 4 in Pond system and 32.75% AR in water phase at day 3 in River system), the amount of not identified radioactivity (NIR) decreased in both systems continuously until finalisation of the study (17.78% AR in Pond system and 2.43% AR in River system). Up to six metabolites could be detected (HPLC) in the time range (Retention time) of 1.8 to 3.2 minutes. The applicant did not manage to separate and identify these metabolites in this study. In sediment phase, not identified radioactivity always remains below 10%.

A second water/sediment study was initiated (Möndel, 2010), in order to better separate, at the analysis step, the metabolites formed in the water phase in a water sediment system, and if necessary identify and quantify the metabolites of concern. For this purpose, natural water/sediment systems were sampled at Kellmetschweiher (Pond in Rhineland-Palatine, Germany) in the same area than the Pond system in the former study. Two replicates were treated with a four-fold higher concentration of ^{14}C-labeled chlorocresol (0.76 mg and 0.78 mg radiolabelled Preventol CMK per litre, respectively) and an appropriate HPLC-method capable of splitting up and quantifying the metabolites of ^{14}C-Preventol CMK. Maximum formation of NIR was measured in one replicate six days after treatment and amounted to 23.9% AR. The test was stopped seven days after application (NIR = 22.8% of AR) and the water phase was collected and used for further characterisation of NIR. The HPLC analysis of the separated and concentrated NIR revealed up to seven base line separated peaks. The retention times of the seven distinct metabolites range between 1.1 and 18.9 minutes. Short retention times of the 1st and 2nd peak indicate the formation of very polar degradation products after oxidation and/or cleavage of the aromatic ring structure (see supposed metabolic pathway on Figure 5.1-1 and Figure 5.1-2). 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.

The quantification of the areas of each peak indicated that the maximal peak area was measured for the 4th peak, with 30.3% of the NIR measured at 7 days, corresponding to a maximum of AR of 6.9%. By carrying out co-chromatography, identical retention times were determined for 4th peak and phenol.

Assuming that same metabolites should have been formed in the same proportions in the former water sediment study leads to the conclusion that only phenol could be a metabolite of concern, with a maximum of 9.9% AR at days 3 in the river system and 8.2% at days 4 in the pond system. The threshold of 10% seems not to be achieved. However, since information dealing with the environmental fate and the ecotoxicology of phenol are available in the Risk Assessment Report, they will be briefly presented in this document.

Two supportive studies on the aerobic aquatic degradation of the compound are available and will be summarized below.

The microbial degradation of CMK (100 and 200 mg CMK/L) was investigated in Rhinewater samples, incubated at 28°C (Rast and Kölbl, 1987). In a second step, two bacteria strains (RST 160-1 and 160-2) were isolated from each of the concentrations by culturing with CMK as the sole carbon source. Strain 160-1 was further cultured with CMK and the mineralisation of various phenol and pyrocatechol derivatives was determined.

When CMK was added to Rhinewater, complete degradation was found after 14 days for the 100 mg/L concentration. At 200 mg/L, complete degradation of CMK was observed after 28 days for two replicates, whereas only 10% and 50% of CMK was degraded in the two other replicates. However such high concentration of CMK is not expected in surface water. Two bacteria cultures were isolated from each of the different initial dosages which had led to a complete degradation. Both strains were able to grow with CMK as the sole carbon source. The test substance was quantitatively degraded and dechlorinated after 30 hours (strain 160-1) and 48 hours (strain 160-2). The degradation experiment of different phenol and pyrocatechol

derivatives by strain 160-1 (cultured with CMK as the sole carbon source) revealed the oxidation of phenol, various cresols and chlorocresols. Among the chlorophenol isomers only 4-chlorophenol was oxidised, however, at a low rate. Pyrocatechol, 4-methylpyrocatechol and 4-choropyrocatechol were reacted at rates 10 to 15 higher. The presumed intermediate of the degradation of CMK, i.e., 3-methyl-4-chloropyrocatechol, was oxidised to a high extent and had a considerable higher oxidation rate than the test compound.

The applicant has submitted a specific study where the degradation of CMK (5 and 10 mg/kg) was investigated over 8 days in a washing water sample received from a poultry stable where CMK-containing disinfectant products are applied (Gerharz, 2011b). HPLC analysis could not detect any CMK residues in the washing water received from the poultry stable. The washing water was then well mixed and spiked with CMK solution in isopropanol into open glass bottles. After mixing the samples, the initial concentration of CMK was determined by HPLC analysis. For each sampling event (day 0, 1, 3 and 8), the samples were centrifuged and the supernatant was used for CMK analysis by HPLC. Duplicate analytical results indicate 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. This study has not been performed according to an adequate guideline and criteria usually investigated in such studies are missing (recovery, degradation of a reference substance, control sample, control sample with the solvent, temperature…). Therefore, the RMS considers this study as supportive data.

Two monitoring reports performed by Bayer in 1987 and 1989 in indicate a low concentration of CMK in the river (below 0.1 $\mu g.L^{-1}$) whatever the location of the sampling (before or after the Bayer plant and a STP).

At last, bibliographical monitoring data in surface water and sediment, investigated in Germany, United Kingdom and Portugal have been provided.

In Germany, several analyses have been carried out in the Baden-Württembert region (South West of Germany).Sampling occurred in June 1998 for the surface water and between 1996 and 1999 for the sediment. CMK has only been detected (below the quantification limit of 0.010 $\mu g.L^{-1}$) in the river Körsch which receives effluents from 6 STPs (Bolz et al., 2001; Körner et al., 2001). The highest sediment concentration (15 $\mu g.kg^{-1}$) was also measured in the river Körsch. It was detectable in the sediment samples from 4 of 6 of the other rivers investigated, mainly at concentrations equal or below 2 $\mu g.kg^{-1}$. At last, CMK was not present above the detection limit in Lake Constance sediment (Bolz et al., 1999). Schmidt-Bäumler et al. (1999) collected 30 representative surface water samples from sewers, rivers, canals and lakes in Berlin in 1996. Samples were taken above and below points where sewage effluents were discharged into the surface water. CMK was found in 22 of the 30 water samples at concentrations between 0.05 $\mu g.L^{-1}$ and 0.14 $\mu g.L^{-1}$. A 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. Considering surface waters, 96% (n = 1596) of all analysed samples did not contain CMK above the limit of detection (LOD = 0.2 to 5 $\mu g.L^{-1}$). In the remaining surface water samples (n = 66), CMK concentrations varied between 0.5 – 6.9 $\mu g.L^{-1}$. In almost half (n = 32) of the samples containing CMK, residues were below 1.0 $\mu g.L^{-1}$. The high measured concentrations resulted from different diffuse contaminations (steel works, urban residential and industrial estates, sewer overflows, public highways, old mining areas, an underground fire. Problems during the installation of a new effluent treatment plant at a paper mill). Groundwater samples did not contain CMK above the LOD. In the case of marine water, CMK was greater than the LOD in only one sample (0.6 $\mu g.L^{-1}$, 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. CMK was detected in 64 of 632 surface water samples (LOQ $\leq$ 0.1 $\mu g.L^{-1}$), and over 0.1 $\mu g.L^{-1}$ in 52 samples, corresponding to 8.1% of total samples. In 49 of these samples the substance was present at

concentrations between 0.1 and 1.0 µg.L^{-1} whereas 2 samples contained the compound at a higher concentration than 1.0 µg.L^{-1}.

In summary, the monitoring data support the argument that CMK will not persist in aquatic systems.

Table 5.1-1: 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 concentratio n (µg/kg d.m.)	Reference
River Rhine. Before and after a STP and a Bayer plant (Germany)	Mai June 1987 Nov. – Dec 1989	0.005-0.1 (LOD)	0.009- <0.1	n.m.	Grote (1987) Oblak (1989)
Sewers, rivers, canals and lakes - Berlin area (Germany)	Sept.. 1996; n = 30	0.015 (LOD) 0.050 (LOQ)	n.d. (n=8) 0.05-014 (n= 22)	n.m.	Schmidt-Bäumler et al. (1999)
2 rivers, one lake - Baden-Württemberg (south-west Germany)	n.r., n = 3	n.r.	n.m.	2 (n = 1) n.d. (n= 2)	Bolz et al. (1999)
8 rivers - Baden-Württemberg (south-west Germany)	Sw: June 1998; n = 23 Sed: 1996-1999: n = 3	Sw: <0.01–0.05 (LOD)/ 0.01 – 0.05 (LOQ) Sed: ≤ 0.5 (LOD)/ 0.6 – 2 (LOQ)	< 0.010 (n=1) n.d. (n=22)	1 – 15 (n=3) 2 (n=2) 1 (n=2) n.d. (n=4)	Bolz et al. (2001), Körner et al. (2001)
Rivers and brooks - Midland and North West regions, UK	1995; n = 1662	LOD = 0.2 - 5	n.d. (n = 1596) < 1.0 (n = 32) 1.0 – 6.9 (n = 33)	n.m.	Dixon (1997)
Groundwater - 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

Conclusion:

In water/sediment systems chlorocresol rapidly dissipated from the whole system with a DT_{50} lower than 2 days at a temperature of 20°C (DT50 ≤ 3.6d at 12°C). The amount of not identified metabolites could reach 27 to 33 %, with a DT_{50} of 7 to 36 days in the water compartment. A second study performed in same conditions with a more efficient analytical method allowed stating that only phenol could be considered as a relevant metabolite, with 9.9% of the applied radioactivity. Supportive data confirm the fast degradation of CMK in aquatic medium and a test performed with isolated strains of bacteria from Rhine water brought some additional insights about the metabolic pathways of CMK.

Aerobic degradation in soil

No key study was submitted by the applicant on the aerobic degradation in soil but two publications and an additional supportive study were provided dealing with the degradation of CMK under laboratory conditions in soils. Those studies did not include an evaluation of specific chemical loss mechanisms (no abiotic control, no gaz production recording, no information concerning recovery). Additionally, the formation of metabolites of concern and of non extractable residues which could have occurred in the case of a no complete degradation has not been investigated. A complete degradation study of CMK in soil, providing identity and quantification of the potential metabolites of concern, and quantification of non extractable residues, has not been provided but is not required because of the ready biodegradability of CMK. Nonetheless, bibliographical information dealing with the metabolism of phenol and phenolic compounds are reported in this document.

Sattar (1989) investigated the degradation of CMK in two soils, i.e., a silty clay (pH 5.0, organic matter 3.5%) and a sandy clay (pH 7.1, organic matter 1.1 %). The soils were treated at rates of 0, 10 and 1000 mg/kg of non radiolabelled CMK and incubated at 25±2°C in the dark for 40 days.

At termination of the test (after 40 days), 22 % (mean of both soils) of the applied CMK was still detectable in the soils. Single first order DT_{50} values accounted for 21 days (equivalent to 59.4 days at 12°C).

Loehr and Matthews (1992) determined the loss rate of CMK when applied to an acidic sandy loam (pH 4.8, organic carbon 0.94 %) and a basic sandy silt loam (pH 7.8, organic carbon 3.25 %). The non radiolabelled test substance was applied at 10 mg/kg and 20 mg/kg to the acidic and the basic soil, respectively. The samples were maintained at a moisture content of 80 % field capacity in the dark at 20°C for 64 days.

In the acidic soil a first-order half-life of 4.2 days (zero order loss rate of 0.9 mg/kg/d) was found while in the basic soil the first order half-life amounted to 1.4 days (zero order loss rate of 3.8 mg/kg/d).

In the supportive study (Nitsche, 2011), 10 mg/kg of non radiolabelled CMK was added to a sandy silt loam soil (pH 6.9, total organic matter 1.9%). The treated soil samples were incubated at 22-23°C in the dark for 19 days. The degradation half- life was 5.4 days under the test condition (equivalent to 12.5 days at 12°C). Because of the several deficiencies (test carried out with a lonely soil, no microbial biomass measurements, no mass balance determination and no investigation dealing with potential formation of metabolites of concern and not extractable residues), this test has only been considered as supportive by the RMS.

As CMK, phenol has been shown to be readily biodegradable (Summary Risk Assessement Report, Joint research Center of the European Commission, 2006) and the degradation pathway of phenol is well known (see Figure 5.1-1). Additionally, a literature study (Federle et al 1988) showed that when phenol is applied in soil sample, mineralization rates over 5% occurred, indicating that the unacceptable conditions dealing with the non extractable residues and mineralization lever are not fulfilled. Moreoever, Weijnen et al. (1989) have shown that the mineralisation of the 4-chlorophenol (structurally related compound to chlorocresol regarding the para-position for the chloride) was over 5% (14.7-20% after 10 days) except for the very alkaline soil pH (8.8). Similar results have been reported by Haider et al. (1973) more than 15% of mineralization after 3 days of incubation of 4-chlorophenol with a neutral soil. These results indicate that chlorination of phenol in para-position do not deal to fulfill unacceptable persistency criteria for the soil. In conclusion, these information support that CMK will not persist in soil.

Conclusion:

No key study (including investigations of potential metabolites of concern and not extractables redidues) has been carried out. However the substance has been shown to be readily biodegradable and according to provided studies, CMK can be expected to dissipate when unintentionally reaching the soil compartment (estimated laboratory half-lives between 2.7 and 59.4 days at 12°C).

5.1.3 Summary and discussion of degradation

Water

The test substance is hydrolytically stable under environmental pH conditions. As the UV absorbance of the molecule is below the solar irradiation at the earth's surface, direct photodegradation is unlikely under environmental conditions.

CMK is readily biodegradable and inherently biodegradable under stringent test conditions.

In water/sediment systems chlorocresol dissipated from the whole system with a DT_{50} lower than 2 days at a reference temperature of 20°C (DT50 $\leq$ 3.6d at 12°C). The amount of not identified metabolites could reach 27 to 33 %, with a DT_{50} of 7 to 36 days in the water compartment. The different metabolites were not identified in this study, however a second study performed in same conditions with a more efficient analytical method allowed stating that only phenol could be considered as a relevant metabolite, with 9.9% of the applied radioactivity.

Soil

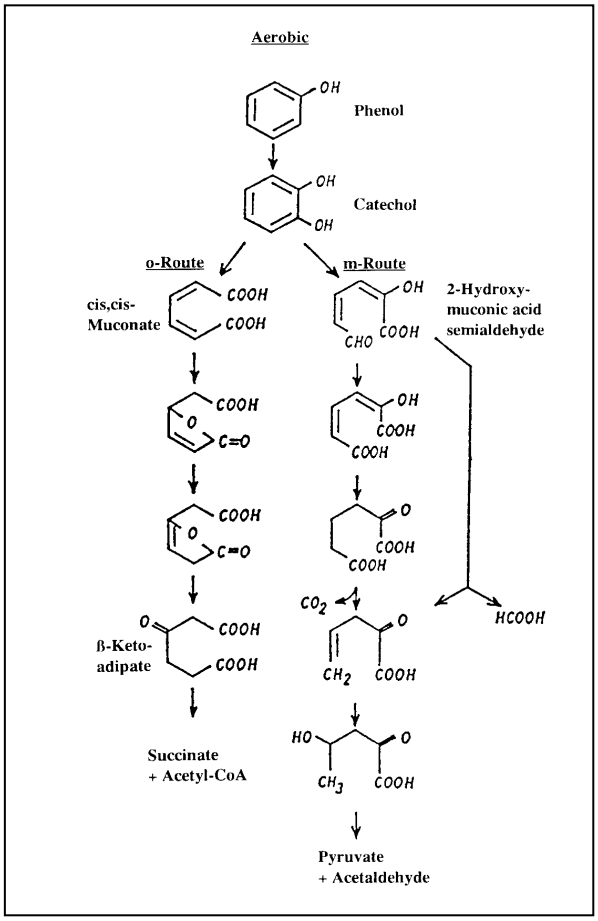
Supportive data indicate a fast to a moderate dissipation rate of CMK in soil (from 2.7 to 59.4 days at 12°C). However, no key study, carried out according to suitable guideline, has been provided.

Metabolism pathways

Some insight about the metabolic pathways of CMK have been brought. In the water sediment study the lonely identified metabolite is phenol with a maximum of 9.9% of initial applied radioactivity. The other transient degradation products are assumed to go through a hydroxylation of their methyl group followed by oxygenation to aldehydic and carboxylic structures. None of the transformation products build in the aquatic environment reached the trigger value of 10% of the parent substance and the half-lives for the degradation products of *p*-chloro-*m*-cresol ranged between 13 and 72 days (calculated at 12°C).

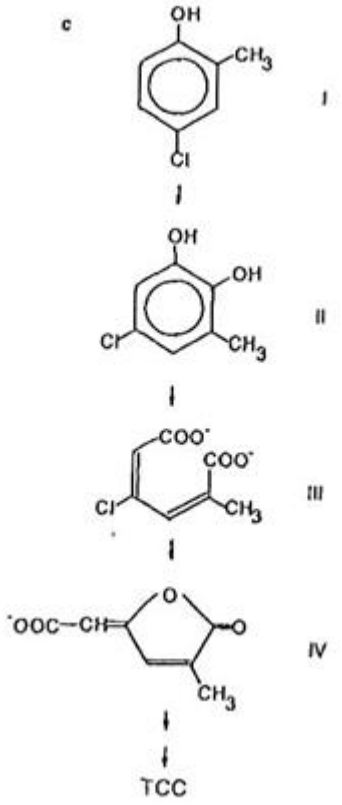
The generation of phenol may be explained by a dehalogenation step in the paraposition of the aromatic ring and an oxidative/reductive elimination of the methyl side chain. Phenol has been shown to be ready biodegradable (Summary Risk Assessement Report, Joint research Center of the European Commission, 2006). Additionally, the degradation pathways of phenol in environment have been well described in literature (Phenol, BUA Report 1997): phenol is degraded under aerobic conditions via an ortho- or a meta-cleavage of the aromatic ring via catechol intermediates of the central metabolism. An illustration of the breakdown pathway of phenol is given in Figure 5.1-1. The degradation starts with an initial oxidation step, followed by cleavage of the aromatic ring structure, which results in carbonyl and carbonic acid structures. These polar substances (e.g. aldehyde, ketone, carboxylic acid etc.), which can be regarded as transient degradation products, are rapidly further degraded either to CO_2 or react chemically with the organic matter in the soil or sediment. The resulting bound residues should afterwards be slowly stepwise degraded to CO_2.

Figure 5.1-1: Aerobic biodegradation pathways of phenol (BUA Report 209, 1997, p 142)



Another degradation pathway is proposed by Rast and Kölbl (1987) in a degradation study of CMK in Rhine water. Rast suggested that CMK follows the same degradation pathway than phenol, e.g. via a cleavage of the aromatic ring via methyl-chlorocatechol intermediates of the central metabolism. Such degradation pathway has been demonstrated for an isomere of CMK, the 4-chloro- 2-cresol by Lechner et al. (1995). In the water degradation study, Rast showed that the presumed intermediate 3-methyl-4-chlorocatechol was oxidised by bacteria via meta-cleavage at a 7-fold higher level than CMK, indicating that the degradation of the presumed metabolite will be faster than those of the parent susbtance.

Figure 5.1-2: Aerobic biodegradation pathways of an isomere of CMK, the 4-chloro- 2-cresol (Lechner et al, 1995). **I: 4-chloro- 2-cresol; II: 5-chloro-3-methylcatechol; III: 4-chloro-2-methyl-cis,cis-muconate; IV: 2-methyl-4-carboxymethylenebut-2-en-4-olide**.



In conclusion, according to the water sediment study and the assumption from the degradation study of CMK in Rhine water, two metabolic pathways of CMK are assumed in the aquatic compartment, via the phenol as first metabolite or via a chloro-methyl-catechol as first metabolite. In the absence of a complete degradation study of CMK in soil, similar degradation pathways than in the aquatic compartment are assumed for the terrestrial compartment. According to the Risk Assessment Report for phenol, this metabolite is readily biodegradable and should not require additional evaluation in the CMK dossier. Chloro-methyl-catechol seems not to be readily biodegradable according to biowin 4.10 (run with the 5-chloro-3-methylcatechol). Nonetheless, the oxidation rate of the 3-methyl-4-chlorocatechol has been shown to be higher than this of CMK, indicating that the degradation of the presumed metabolite will be faster than those of the parent susbtance.

Non extractable residues

The formation of not extractable residues has been investigated in the water sediment systems. The studies indicated rather high amounts of not extractable residues (until 54% and 46-52% at the end of the study). Nevertheless, the threshold of 70% has not been achieved and the minerealisation was over 5% (24-37%).

A complete degradation study of CMK in soil is not avalaible. However, similar degradation pathways of CMK in the aquatic and the terrestrial compartment is assumed, and read across performed with phenol and phenolic compounds, as well as the ready biodegradability of the substance indicate that CMK will not persist in soil.

5.2 Environmental distribution

Method	Results					Remarks	Reference
OECD guideline 121 (proposal for new test guideline 121, 2001)	Absorbed n.a.	K_a 1	K_{aOC} 2 158.5	K_d 3	K_{dOC} 4		Erstling and Feldhues, 2001 b
Adsorption/ desorption test OECD Guideline No. 106 and Commission Directive 2001/59/EC, Method C.18. Soil 1 : Sand Soil 2 : Loamy sand Soil 3 : Clayey loam Soil 4 : Loam-	Absorbed a.s. [%] Not given	K_a 1 1.9 11.8 9.3 7.6	K_{aOC} 2 160.9 497.9 508.2 230.3	K_d 3 Not given	K_{dOC} 4 Not given		Meinerling, 2007

1 K_a = Adsorption coefficient
2 K_{aOC} = Adsorption coefficient based on organic carbon content.
3 K_d = Desorption coefficient).
4 Kd_{OC} = Desorption coefficient based on organic carbon content
n.a.: not applicable

5.2.1 Adsorption/Desorption

The study by Erstling and Feldhues (2001 b) investigated the adsorption of CMK in a screening test using High Performance Liquid Chromatography (HPLC). The test was performed according to OECD guideline 121 (proposal for new test guideline 121, 2001). Six reference standards of known Koc were analysed by HPLC to determine their retention times. Sodium nitrate was used to define the system's dead time (t_0). From the retention times and the dead time of the HPLC column, the capacity factor (k') of each substance was calculated and a plot of the tabulated log Koc values of the calibration substances versus log k' (measured) was developed using linear regression.The linear regression of log Koc values against measured log k' 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 estimated log Koc value for CMK was 2.2, corresponding to a Koc of 158.5.

The study by Meinerling (2007) investigated the adsorption behaviour of CMK in a batch equilibrium test with four soils according to OECD Guideline No. 106 and Commission Directive 2001/59/EC, Method C.18. In a preliminary study the soil/solution ratio as well as the equilibrium time, the potential adsorption of CMK on surfaces of the test vessels and the stability of the test item were estimated. Two soils and three soil to solution ratios (1:1, 1:5 and 1:25) were used in a preliminary test. In a screening test the adsorption kinetic of CMK at four different soil types was studied using a soil to solution ration of 1:2.5, a single concentration (approx. 10 µg/mL) and determining the distribution coefficients K_d and K_{oc} after different time points (8, 24, 48 h). The desorption kinetics were performed to investigate whether the test item was reversibly or irreversibly adsorbed on the soil. An equilibrium time of 48 h was considered for the screening test. CMK 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. The results are presented in **Erreur ! Source du renvoi introuvable.**. 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 of 160.9 to 508.2 mL/g. Desorption of adsorbed CMK from the different soil types ranged from negligible desorption up to 22% desorption after an equilibrium time of 48 h.

In this study mass balances range from 16.7% to 102.2% (lower than 90% for all the soils except Loam soil). A complementary report has been provided by the applicant on the stability of the molecule during the experiment showing that mass balance deficiencies in the study can be explained by an insufficient extraction step and not by the instability of the test substance (Meinerling, 2008). This explanation was accepted. Nevertheless in order to avoid an underestimation of release to groundwater, a mean of the two lowest Koc values (195.6 mL/g) was considered as endpoint. In fact, the two lowest Koc values have been obtained for the sand and the loam soils, for which the mass balances were almost high (77% for sand and 98% for loam) whenever mass balances were lower than 30% for loamy sand and clayey loam. Moreover this value is close to those found with HPLC method (158.5 mL/g) and in the reference below.

Ohlenbusch et al. (2000) investigated the sorption of CMK to dissolved organic matter by solid phase microextraction (SPME). For the sorption to a commercially available humic acid a log Koc of 2.47 (Koc = 295) was determined.

Conclusion:

The results of the HPLC screening test (Koc = 158.5) as well as the Koc values obtained for CMK from the batch equilibrium experiment (160.9 to 230.3 mL/g, arithmetic mean: 195.6 mL/g), reveal CMK to be of moderate mobility in soils

5.2.2 Volatilisation

The tropospheric half-life of CMK was estimated using the AOPWIN program (A7.3.1/01). The software (US-EPA, 2000, version 1.92) is based on a quantitative structure analysis developed by Atkinson. The calculation method sums up the reactivity of all structural elements towards OH radicals. Using a 24-hours day and a mean daily OH concentration in air of 0.5×10^6 OH radicals per cm^3, a half–life in air of 0.625 days was assessed – corresponding to a chemical life-time in air of 14.995 hours (Anthe, 2006).

5.2.3 Distribution modelling

Not performed.

5.3 Aquatic Bioaccumulation

Table 22: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
USEPA - N, 165-4 Flow through	BCF = 321 L/kg (whole fish)	Log K_{ow} = 3.5-4.0 Metabolites : MB045950, MB046136: rapidly eliminated Initial concentration of a.s.: 0.85 µg/L Depuration time >99% eliminated from whole fish within 14 days.	Chapleo, S. Hall, B. E. (1992)
TGD for Risk assessment part 2 section 3.8.3.2	BCF=501	Calculated value from log Kow=4	Chabassol Y Reynaud R (1991)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The chemical risk for bioaccumulation in aquatic species can be assessed by evaluating the bioconcentration factor (BCF) which consists of the ratio between the concentration of a substance in the organism and the corresponding concentration in water at steady state conditions. As a first step, the BCF for CMK can be estimated on the basis of the partition coefficient n-octanol/water (equation 74 of the Technical Guidance Document on Risk Assessment (EU, 2003) according to the equation (Veith *et al.*, 1979):

$\log BCF_{fish} = 0.85 \times \log K_{ow} - 0.70$

K_{ow} = octanol/water partition coefficient
BCF_{fish} = bioconcentration factor (L x $kg_{wet\ fish}$)

For CMK a log K_{ow} value of 3.02 at 22 ± 1°C has been determined). Using the TGD equation and this log K_{ow} value, a log BCF_{fish} of 1.867 (BCF_{fish} = 73.6 L/kg) can be calculated, which does not give a lead for a bioaccumulation potential of the substance (Paul, 2007).

5.3.1.2 Measured bioaccumulation data

The result of the calculation is in the same order of magnitude as the outcome of a bioaccumulation test conducted according to OECD Test Guideline 305C with the carp (Cyprinus carpio). At test concentrations of 2 and 20 µg CMK/L bioconcentration factors of 5.5 - 11 (log BCF = 0.7 – 1.0) and 6.7 – 13 (log BCF = 0.8 – 1.1) were obtained, respectively (MITI, 1992). This study comes from the Japoneese database on existing chemicals, elaborated by the Japoneese Ministry of International Trade & Industry. An insufficient description of test system the test procedure and of the results and observations is provided. Therefore, the BCF value determined in this study was not accepted but is considered as supportive of the BCF estimation using QSAR-approach.

Jennings et al. (1996) investigated the bioconcentration of several phenolic substances in two marine species, i.e. the mussel *Mytilus edulis* and the carnivorous fish *Trachurus novaezelandiae*. The experimental set-up included maintenance of the test organisms at constant temperatures (15°C for the mussels and 21°C for the fish), three test concentrations (1, 10 and 100 µg/L), three replicates per organism and test concentration, and one week of exposure with sampling after 1 h, 6 h, 12 h, 24 h, 48 h and one week. After one week of exposure remaining animals were transferred to clean water and sampled after 1 h, 6 h, 12 h, 24 h and 48 h.

Only the individuals exposed to a concentration of 100 µg CMK/L accumulated the test substance above trace amounts. Bioaccumulation at this test concentration proceeded rapidly, i.e., a plateau concentration was already reached after 6 h (mussels) and 24 h (fish). The fish accumulated to a higher extent than the mussel however, residues could only be detected in the muscle tissue and not in the livers. After transfer to clean seawater CMK was depurated rapidly with only traces remaining after 12 h. The bioconcentration factors has been calculated for the test organisms when a steady state was reached and amounted to 38 (mussels, log BCF = 1.6) and 121 (fish, log BCF = 2.1).The values determined in this published study are thus in good accordance with the BCF calcultated through the QSAR approach.

5.3.2 Summary and discussion of aquatic bioaccumulation

Bioconcentration factors for the aquatic compartment obtained by calculation or experimentally derived vary between 5.5 and 120.8. Only one of 6 experimentally obtained values exceeded a BCF value of 100. The BCF_{fish} of 73.6 L/kg defined by calculation is considered as acceptable and indicates a low potential of CMK to bioaccumulate.

5.4 Aquatic toxicity

Table 23: Summary of relevant information on aquatic toxicity

Method			**Results (mg a.s./L)**	**Remarks**	**Reference**
Secondary consumers	Acute toxicity to **fish**	(*Oncorhynchus mykis* - 96h LC50) US EPA FIFRA 72-1	LC_{50} = 0.92 (mmc)	SS	Gagliano and Bowers, 1993 a RI = 2
	Chronic toxicity to **fish**	(*Oncorhynchus mykis*- 28d NOEC)	NOEC = 0.15 (mmc)	SS	Schneider and Wydra,

		OECD 204 (1984) + 215 (2000)			2007 RI = 2
		(Brachy-danio rerio - 14d NOEC) Comparable with OECD 204 (1984)	NOEC = 1.0 (nc)	F	Caspers and Müller 1991 and Weyers, 2006 c RI = 1
Primary consumers	Acute toxicity to **freshwater invertebrates**	*(Daphnia magna* – 48h EC50*)* U.S.-EPA FIFRA § 72-2	EC50= 2.29 (mmc)	S	Gagliano and Bowers, 1993 b RI = 2
	Chronic toxicity to **freshwater invertebrates**	*(Daphnia magna* – 21d NOEC) EEC – C20 OECD Guideline No. 211 (1998)	NOEC= 0.32 (nc)	SS	Weyers, 2007 RI = 2
Primary producers	Toxicity to **freshwater algae and aquatic plants**	*(Desmodesmus subspicatus* – 72h) OECD Guideline No. 201	E_rC_{50} = 30.62 (nc)* NOE_rC = 9.8(nc)*	S	Vinken and Wydra, 2007 RI = 1

S: Static; SS: Semi-static; F: Flow-through;
R1/R2 : reliability of the study
mmc: mean measured concentration; nc = nominal concentrations
* analytical measurements showed 93 % recovery of initial concentrations during the test period, thus the nominal concentrations were used as results

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

A 96 - hour static-renewal study (Gagliano and Bowers, 1993a) 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*) at the initial measured concentrations of 0.218, 0.366, 0.644, 1.110 and 2.039 mg a.s./L. No mortalities or any symptoms of intoxication occurred in the control groups (dilution water and solvent control). 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 over the two first days in the lack of measurement of test substance at 72h and 96h. The validity criteria of the test can be considered as fulfilled and the result will be used in the risk assessment.

5.4.1.2 Long-term toxicity to fish

The long-term toxicity of Preventol CMK has been tested in a juvenile growth test with the Rainbow trout (*Oncorhynchus mykiss*) according to "OECD Guideline for Testing of Chemicals, Section 2, No. 204: "Fish, Prolonged Toxicity Test: 14-day Study", adopted April 04, 1984 and No. 215: "Fish, Juvenile Growth Test", adopted January 21, 2000 (Schneider and Wydra, 2007). Ten juvenile Rainbow trouts were exposed to nominal concentrations of 0.019, 0.061, 0.20, 0.63 and 2.0 mg Preventol CMK/L (corresponding to mean measured concentrations of 0.012, 0.044, 0.15, 0.50 and 1.9 mg/L) and a control for 28 days. As the mean recovery rate in the freshly prepared test concentrations was 96% (average for all concentrations) and 51% in the aged test media (average for all concentrations), the results are reported related to analytical mean concentrations of the test item. The daily recorded effects were mortality and symptoms of intoxication. Furthermore, at the

start and the end of the test, the growth parameters body weight and length of surviving fish were determined. The resulting NOEC based on pseudo specific growth rate was 0.15 mg test item/L. The LOEC was determined to be 0.5 mg test item/L.

Additionally, the prolonged toxicity of CMK to Zebrafish (*Brachydanio rerio*) was tested (Caspers and Müller 1991 and Weyers, 2006 c). The test was carried out based on the method described by UBA-Draft method: "Prolonged Toxicity Test with Zebrafish – *Brachydanio rerio* (1984)". This method is comparable to OECD Guideline 204 "Fish, Prolonged Toxicity Test: 14-day study" (adopted 1984). Chemical exposures were conducted for 14 days, mortality and abnormal behaviour was recorded daily. Based on the observed abnormal behaviour and death of all fishes in the highest concentration and no observed effects in the treatment below the NOEC as 1.0 mg/L. Results were based on nominal concentrations, due to mean measured concentrations from 104 to 105 % at the NOEC and the highest concentration, respectively.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Juvenile *Daphnia magna* were exposed in a static test system for 48 h to five concentrations of Preventol CMK (1.0, 2.0, 3.0, 4.0 and 5.0 mg a.s./L) according to U.S.-EPA FIFRA § 72-2: "Acute toxicity test for freshwater invertebrates" (Gagliano and Bowers, 1993b). Mortality of daphnids showed a clear dose-response relationship: no daphnids died in the lowest concentrations, while no *Daphnia* survived in the highest concentration. The EC_{50} value based on immobilisation and sublethal effects was determined to be 2.29 mg a.s./L after 48 h. Concentrations were measured at day 0 and at day 2 and range between 87 and 110 % of nominal, indicating that the test substance was stable for the duration of the study. All results based on mean measured concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

The long-term toxicity of Chlorocresol has been tested in *Daphnia magna* reproduction test (semi-static conditions) according to "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)) (Weyers, 2007). Ten *Daphnia* per concentration were exposed to nominal concentrations of 0.01, 0.032, 0.10, 0.32 and 1.0 mg Chlorocresol/L and a control for 21 days. The concentrations of CMK have been measured in the lowest, the medium and the highest tested concentrations. The recovery rates in the freshly prepared test concentrations and old media were above 80% for the medium and the highest tested concentrations (1 and 0.1 mg/L). The mortality of adults and the number of neonates was observed three times per week at the renewals of the test media. Results based on nominal concentrations showed that 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.

Another long-term toxicity data has been performed according to a German Guideline, obtained a NOEC of 1.25 mg a.s./L related to the nominal concentration of the substance as this was defined to be > 80% of the measured concentration in the solution containing CMK but no daphnia. However a lower recovery (60%) occurred at 19 days in the test solution containing daphnia. This study is therefore considered as supportive data (Kühn et al, 1988 and Jungheim, 2006).

5.4.3 Algae and aquatic plants

A first study has been submitted (Caspers, 1983/1991 and Weyers 2006a) but has been considered as not reliable because of several deficiencies (no monitoring of the test substance, number of tested

concentrations below the guideline recommandations, no pH measurement, higher initial cell density compared to the guideline recommandations…)

Therefore, the influence of CMK on the growth of the green alga Desmodesmus subspicatus (formerly Scenedesmus subspicatus) was investigated in a second study (Vinken and Wydra, 2007) in a 72 hours static test according to the following guidelines: OECD 201 (2006), Directive 92/69/EEC, C.3 (1992).

The calculated average growth rates decreased in a dose dependent manner. The 72 h NOE_rC value was 9.8 mg a.s./L, based on the lack of a statistical growth inhibition at these concentrations. Based on growth rate, the 72-hour E_rC_{50} value was 30.6 mg a.s./L. Based on the mean area under the growth curve, the NOE_bC was 3.1 mg a.s./L and the 72-hour E_bC_{50} value was 17.2 mg a.s./L. Mean measured concentrations ranged from 97 % at test start to 89 % at test end of the nominal concentration during the test. Therefore, all endpoints were based on nominal concentrations of the test item. Validity criteria for the acceptance of the test can be considered as fulfilled.

5.4.4 Other aquatic organisms (including sediment)

No study dealing with the toxicity of CMK on sediment dwelling organisms has been provided. The log of Kow is 3, which is the threshold value to require a toxicity test on sediment organism. However, the mean Koc value remains below the threshold value of 500 indicating that the risk assessment for the sediment is not required.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Regarding all available toxicity data, fish are the most sensitive species for acute and chronic effects. These results are used to classify the active substance CMK.

Considering that the 96h-LC_{50} = 0.92 mg/L value was obtained for *Oncorhynchus mykis* is lower than 1 mg/L, CMK meets the criteria for classification as **Aquatic Acute 1** for environmental hazard according to CLP criteria. This value is extracted from a publication for which FR-MSCA considers sufficient information available to be considered. As this value is within the range of 0.1-1.0 mg/L, an **M-factor of 1** is allocated.

Considering that CMK is rapidly degradable and that the 28d-NOEC = 0.15 mg/L value obtained for *Oncorhynchus mykis* is within the range of 0.1- 1.0 mg/L, CMK meets the criteria for classification as **Aquatic Chronic 3** for environmental hazard according to CLP criteria.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

According to CLP Regulation criteria:

Classification:
Aquatic Acute 1; H400
Aquatic Chronic 3; H412
Acute M-factor: 1
Chronic M-factor: -

Labelling:

Pictogram: 
Signal word: Warning
Hazard statements: H412: Harmful to aquatic life with long lasting effects

6 OTHER INFORMATION

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8 ANNEXES