Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at Community level of

Chlorophene

EC Number: 204-385-8
CAS Number: 120-32-1

CLH-O-0000001412-86-58/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

12 March 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Chlorophene

EC Number: 204-385-8

CAS Number: 120-32-1

Index Number:

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Chlorophene IUPAC name: 2-Benzyl-4-chlorophenol
EC number:	204-385-8
CAS number:	120-32-1
Annex VI Index number:	Not in Annex VI
Degree of purity:	Minimum degree of purity 96,7% w/w
Impurities:	Impurities are not present at concentrations that affect the Classification and Labelling of this substance. Detailed information about the impurities is presented in the confidential part of IUCLID.

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	Not in Annex VI
Current proposal for consideration by RAC	**Acute Tox 4** H332: Harmful if inhaled **Skin Irrit 2** H315: Causes skin irritation **Skin Sens 1A** H317: May cause an allergic skin reaction **Eye Dam 1** H318: Causes serious eye damage

	STOT RE1 H372: Causes damage to kidneys through prolonged or repeated exposure **Carc 2** H351: Suspected of causing cancer **Repr 2** H361f: Suspected of damaging fertility **Aquatic Acute 1** H400: Very toxic to aquatic life M-factor: 1 **Aquatic Chronic 1** H410: Very toxic to aquatic life with long lasting effects M-factor: 100
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	

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	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	Conclusive but not

					sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox 4 H332 Harmful if inhaled	Not applicable	Not classified	
3.2.	Skin corrosion / irritation	Skin Irrit 2 H315 Causes Skin irritation	Not applicable	Not classified	
3.3.	Serious eye damage / eye irritation	Eye Dam 1 H318 Causes serious eye damage	Not applicable	Not classified	
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.4.	Skin sensitisation	Skin sens 1A H317 May cause an allergic skin reaction	Not applicable	Not classified	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc 2 H351 Suspected of causing cancer	Not applicable	Not classified	
3.7.	Reproductive toxicity	Repr 2 H361f Suspected of damaging fertility	Not applicable	Not classified	
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE1 H372 Causes damage to kidneys through prolonged or repeated exposure	Not applicable	Not classified	
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 H400 Very toxic to aquatic life Aquatic chronic 1 H410 Very toxic to aquatic life with long	M-factor of 1 M-factor of 100	Not classified	

		lasting effects			
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification [3)]

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
[3)] Not listed on Annex I of Regulation (EC) No 1005/2009 on substances that deplete the ozone layer

Hazard statements:

H332: Harmful if inhaled

H315: Causes skin irritation

H317: May cause an allergic skin reaction.

H318: Causes serious eye damage

H372: Causes damage to kidneys through prolonged or repeated exposure

H351: Suspected of causing cancer.

H361f: Suspected of damaging fertility

H400: Very toxic to aquatic life

H410: Very toxic to aquatic life with long lasting effects

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Chlorophene is not included in Annex VI of the CLP regulation.

2.2 Short summary of the scientific justification for the CLH proposal

Concerning physico-chemical properties, chlorophene does not fulfil the criteria for a classification according to Regulation (EC) No 1272/2008 (CLP). Therefore no classification is required regarding physico-chemical hazards.

The active substance is harmful by inhalation and irritating to skin. It presents risk of serious damage to eyes and may cause sensitisation by skin contact. It cause damage to kidneys through prolonged or repeated exposure and is suspected of causing cancer. It is suspected of damaging fertility. It is very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment.

2.3 Current harmonised classification and labelling

Chlorophene is not included in Annex VI of the CLP regulation.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No justification is needed

Part B.

SCIENTIFIC EVALUATION OF THE DATA

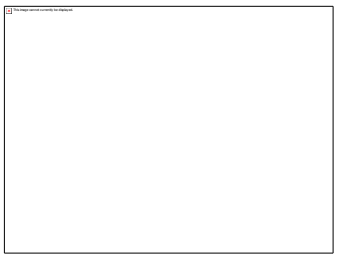
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	204-385-8
EC name:	Chlorophene
CAS number (EC inventory):	
CAS number:	120-32-1
CAS name:	
IUPAC name:	2-Benzyl-4-chlorophenol
CLP Annex VI Index number:	Chlorophene is not included in Annex VI of the CLP regulation.
Molecular formula:	$C_{13}H_{11}ClO$
Molecular weight range:	218.7 g/mol

Structural formula:



1.2 Composition of the substance

Purity/impurities, additives in the active substance

Chlorophene is an active substance with a minimal purity of 95% as specified from the commercial producers. However the 5 batch analyses from the reference source gives a purity of minimum 96,7 % (mean conc. - 3*SD) which will be proposed as purity for the approval decision of chlorophene as an active substance according to the Biocide regulation (EU) No 528/2012.

No impurity reported has been found to be of relevance for the CLP proposal.

The identity of impurities and additives in the active substance chlorophene as manufactured is confidential.

Representative production batches of the active substance are analysed for their chlorophene and impurities content.

In all study summaries information on degree of purity and impurities is given when available (doc III documents). Information on the degree of purity of the material used for most of the studies show a higher degree of purity than that proposed as minimum purity.

1.3 Physico-chemical properties

Table 5: 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	Solid Purity: 97.9 % White to slightly yellow	Kraus, 2006a	Visual assessment
Melting/freezing point	45.9 °C	Jungheim, 2007a	EC method A.1
Boiling point		Jungheim, 2007a	EC method A.2. Up to the decomposition there is no boiling point of the substance. Exothermal decomposition starts at 110 °C.
Relative density	1.317 at 20 °C	Jungheim, 2007a	EC method A.3
Vapour pressure	< 1.0E-03 Pa at 20 °C < 1.0E-03 Pa at 25 °C 1.66E-02 Pa at 50 °C	Olf, 2006	EC method A.4
Surface tension	57.3 mN/m at 20 °C Chlorophene is surface active.	Jungheim, 2007a	EC method A.5
Water solubility	Results at pH 7: 0.083 g/L at 10°C 0.117 g/L at 20°C 0.199 g/L at 30°C Temperature dependence on water solubility was observed. An effect of pH-value is not expected. Comparison with the tested phenolic substance *ortho*-phenylphenol does not deliver any indication.	Jungheim, 2006a and Erstling, 2002	EC method A.6
Partition coefficient n-octanol/water	Results at 25 °C: The log Pow is 4.276 for the unionised species. Log Pow = 4.276 at pH 4 Log Pow = 4.275 at pH 7 Log Pow = 4.175 at pH 9 pH dependence on log Pow was observed. An effect of temperature is not expected. Comparison with the phenolic substance *ortho*-phenylphenol does not deliver any indication.	Greenwood, 2003a; Feldhues, 2006; Erstling, 2002 and Jungheim, 2004	OECD guideline 107
Flash point	-	-	Not applicable as the substance is a solid

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	The substance is not highly flammable.	Heinz, 2007	EC method A.10
Explosive properties	Not an explosive substance	-	Examination of the chemical structure indicates that the substance does not possess any explosive properties.
Self-ignition temperature	-	Heinz, 2007	EC method A.16. The substance gave no exothermic indication up to its melting point.
Oxidising properties	Not an oxidising substance	-	Examination of the chemical structure indicates that the substance does not possess any oxidising substances
Granulometry	Not conducted	-	-
Stability in organic solvents and identity of relevant degradation products	The solubility of chlorophene in methanol and toluene at 10, 20 and 30 °C is > 250 g/L.	Jungheim, 2007c	CIPAC MT 157 CIPAC MT 181
Dissociation constant	pKa = 9.59	Greenwood, 2003a	OECD guideline 112
Viscosity	-	-	Not applicable as the substance is a solid

2 MANUFACTURE AND USES

2.1 Manufacture

Not necessary for biocides.

2.2 Identified uses

Intended biocide use areas:

Product type 2: Disinfectants and algaecides not intended for direct application to humans or animals

The representative product, Remedor with the active substance chlorophene is intended to be used as a heavy duty disinfectant for both professional and private use. Professional use includes heavy duty disinfection of surgery rooms and infectious disease wards as well as small-area use for disinfection of objects as washbasins and toilet facilities in hospitals by professional cleaning personnel. Private use of chlorophene is also restricted to heavy duty disinfection of objects, such as as washbasin and toilet facilities. Professional users may be expected to use chlorophene containing products on a daily basis, while non-professional use occurs more rarely, maybe on a weekly basis.

Product-type 3: Veterinary hygiene

The representative product, Remedor with the active substance chlorophene is intended to be used by professional workers to control pathogenic micro-organisms in industrial poultry barns. Application is performed using powered medium pressure spray equipment which sprays an even

layer across the surfaces to be treated. Disinfection of poultry barns is normally performed once every 6-8 weeks, but the task may be performed by specialised disinfectors who provide cleaning services for animal facilities. These workers may perform this task on a daily basis, and are expected to use personal protective equipment.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 6: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 9			

3.1.1 Summary and discussion of physico-chemical properties

The data on physic-chemical properties are conclusive but not sufficient for classification. Refer to table 9.

3.1.2 Comparison with criteria

The substance does not meet the criteria for classification for physico-chemical properties (please also refer to table 3 and 9).

3.1.3 Conclusions on classification and labelling

The substance does not meet the criteria for classification for physico-chemical properties (please also refer to table 3 and 9).

RAC evaluation of [physical hazards]

Summary of the Dossier submitter's proposal

The Dossier Submitter (DS) did not propose classification for physical hazards. The data on physico-chemical properties did not indicate any concerns and as such chlorophene does not meet the criteria for classification.

Comments received during public consultation

No comments were made regarding this endpoint.

Assessment and comparison with the classification criteria

RAC is in agreement with the DS that classification is not required for physico-chemical hazards. Chlorophene was shown not to be highly flammable in a standard study (EC method A.10) and so does not meet the criteria for classification as a flammable solid. Examination of the chemical structure did not indicate that chlorophene would have any explosive or oxidising properties, therefore chlorophene does **not** meet the criteria for classification as an explosive substance or an oxidising solid.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Table 7: Toxicokinetic and metabolism studies with chlorophene

Route	Method Guideline	Species Strain Sex No/group	Label	Dose levels	Reference
Gavage, Dermal, Intravenous injection (i.v).	Absorption, excretion, distribution, metabolism No guideline Non-GLP	Rat, Fischer 344 ♂, ≥ 3 per group	^{14}C-o-Benzyl-p-chlorophnol ([^{14}C]BCP)	Gavage: 10, 100, 1000 mg/kg bw Dermal: 10 mg/kg bw I.v.: 5, 10, 25 mg/kg bw	Kao & Birnbaum, 1986 A6_2 (1) **KEY STUDY**
Dermal	US-EPA §85-2 (1982) ≅ OECD 427	Rat, Sprague-Dawley ♂, 16 per group	^{14}C-o-Benzyl-p-chlorophnol ([^{14}C]BCP) (5% solution)	0.5, 5, 50 mg /mL 5, 50, 500 µg/cm² ≅ 0.3, 3, 30 mg/kg bw	Confidential, 1994 A6_2(2) **KEY STUDY**

The **absorption, distribution, metabolism and excretion** of ^{14}C-labelled chlorophene were studied in male F344 rats (Kao and Brimbaum, 1986). The test substance was administered via gavage, intravenous (i.v.) injection and dermal application. Three animals were used for each time point in each study. The study included examination of excretion of radioactivity into bile in the i.v. experiment. The recovery of labelled compound ranged between 90.0-101.4% (Doc IIIA6_2(1); Table A6_2-2: Tissue distribution of chlorophene in tissues after different routes of exposure). Clinical signs were not reported in any of the experiments.

Following gavage administration of ^{14}C-o-Benzyl-p-chlorophnol ([^{14}C]BCP) in corn oil at dosages of 10, 100 and 1000 mg/kg bw (single dose), [^{14}C]BCP-derived was almost completely excreted (>92%) in urine and faeces after 3 days. The relative amounts excreted in urine and faeces were highly dose-dependent. Three days after gavage administration of 10 mg/kg bw ~ 24% and 76% [^{14}C]BCP-derived had been excreted in urine and faeces, respectively. The rate of faecal BCP excretion decreased to 41% when the dose was increased to 100 mg/kg bw. However, when the dose given was 1000 mg/kg bw, relative faecal excretion was increased (62%) compared to the mid-dose, but was still lower than in the low dose group (10 mg/kg bw)(Doc IIIA6_2(1); Table A6_2-2: Tissue distribution of chlorophene in tissues after different routes of exposure[a]). For the mid-dose group urinary excretion was the major excretion route, while for the lowest and highest dose group faecal excretion was the major excretion route. The lowest measured urine excretion after 72 h was found in the group given 10 mg/kg by gavage and was 24.5%. In the tissues of these animals 0.68% of the total dose was recovered.

Since the levels in bile were not measured after oral administration in the submitted ADME study, an oral absorption could be estimated based on the lowest urine excretion in addition to the chlorophene levels found in the tissues. An estimation of oral absorption for chlorphene based on only urine excretion and tissue levels in animals given 10 mg/kg bw gavage would be 25%. However, in the 417 OECD guideline for the testing of chemicals (Toxicocinetics) adopted 22 July

2010, it is indicated that "oral and IV administration of test substance and measurement of net test substance present in urine plus expired air plus carcass by each of the two routes" could be used to estimate oral absorption. An estimation of oral absorption for chlorphene based on this assumption would be 70 % ([(0.68+ 24.46)/ (1.31+ 34.9)]*100 % = 69.4 %; figures could be found in the Table 8 below). As the first assumption is assumed to be too conservative and an alternative option is given in the latest version of the OECD 417 guidance, an oral absorption of 70 % was decided for chlorophene.

For the dermal absorption studies, BCP was dissolved in acetone at a concentration of 40 mg/ml, and 10 mg/kg [^{14}C]BCP (50 µl/animal) was applied to 3.9 cm^2 intrascapular area (hair was clipped). A perforated tissue capsule was held over the treated area with cyanoacrylated adhesive. Excretion via faeces and urine were 34.4%, 50.5% and 59% of the applied dose after 1, 2 and 3 days, respectively. Approximately 32 % of the total dose was found at the skin site and 3% were in tissues and skin. The recovery of BCP after dermal exposure was approximately 95% (Doc IIIA6_2(1); Table A6_2-2: Tissue distribution of chlorophene in tissues after different routes of exposure). The major excretion route was also for dermal absorption via faeces. No information about stripping of the skin before measuring the BCP level was included. Based on the levels in urine, faeces and tissues, dermal absorption of chlorophene was approximately 62%.

To examine bilary excretion 5, 10, or 25 mg/kg bw BCP was given by injection into the femoral vein. The rats were anesthetized with sodium pentobarbital and the bile duct was cannulated. Bile was collected for 6 h after treatment. After 5, 10 and 25 mg/kg bw, 87%, 72% and 56% of the total radioactivity appeared in the bile, respectively. The excretion was statistical significally decreased with increasing exposure dose. Also by comparing faecal excretion after 3 d and biliary excretion after 6 h indicated that less BCP-derived radioactivity was excreted in faeces than in bile (54% versus 72%). This is suggested to be caused by a deconjucation of glucoronyl conjugates by beta glucuronidase activity of intestinal microorganisms and the resulting parent BCP and 4 hydroxy-BCP could then be reabsorbed. These findings indicate that BCP is subject to enterohepatic circulation.

To examine distribution, BCP was dissolved in Emulphor:etanol:water (1:1:4) at a concentration of 10 mg/ml and was injected i.v. at 1 ml/kg into a the tail vein. Also for the i.v treatment the major excretion route was via faeces, similar to the excretion after dermal absorption. After 3 days 88% of the dose was excreted. After i.v administration no volatile metabolites or [^{14}C]CO_2 were detected in exhaled air. Three days after treatment, regardless of dose and exposure route, no tissue contained more than 1% of the total administrated radioactivity. The liver contained the highest percentage of the total administrated BCP-derived radioactivity (0.63%) of all tissues. However, the highest concentration (µg BCP/g tissue) of BCP-derived radioactivity was found in the kidney during the whole measuring period. The BCP concentration in the kidneys was almost 15 times higher than in liver (Doc IIA; Table A6_2-3: Pharmacokinetic parameters for the elimination of chlorophene-derived radioactivity). After 72 h post treatment the highest concentration was found in kidney, liver and spleen 1.7, 1.6, and 0.8 % of the total dose per g tissue, respectively. Muscle, fat and skin contained significant BCP-derived radioactivity 15 min post administration, but little remained after 24 h (< 0.1% total dose/g tissue Doc IIIA6_2(1); Table A6_2-4: Tissue distribution of chlorophene in tissues after i.v. exposurea,). After giving 10 mg/kg bw the half-life of the total BCP-derived radioactivity in faeces and urine together was found to be 14 h. In blood it was suggested that two

BCP-derived components were eliminated with a half-lives of 0.7 and 9.9 h (Doc IIIA6_2(1); Table A6_2-5: Pharmacokinetic parameters for the elimination of chlorophene-derived radioactivity).

The major *in vivo* metabolites detected after BCP exposure were glucuronyl conjugates of chlorophene and hydroxy-chlorophene in faeces and urine. Fifteen minutes after exposure to 10 mg/kg bw i.v. 0.5% and 85% of the total BCP-derived radioactivity were excreted in the bile as UDP-glucuronyl conjugate of OH-BCP and BCP, respectively. After 6 h the proportion of OH-BCP UDP-glucuronyl conjugate were increased to 6%, while the proportion of BCP UDP-glucuronyl conjugate was decreased to 36%. The percent of the conjugates out of the total radioactivity excreted in bile at 15 minutes and 6 hours was 20-25% and 1-2%, respectively. Twenty-four hours after i.v. exposure to 10 mg/kg bw 0.4% and 16% of the total BCP-derived radioactivity were excreted in the urine as UDP-glucuronyl conjugate of OH-BCP and BCP, respectively (Doc IIIA6_2(1) Table A6_2-3 Glucuronide conjugates in bile and urine) . The percent of the conjugates of the total radioactivity excreted in urine was between 25-40% after 24 h. Glutathione conjugates were also found in urine. Based on the study results, a metabolic pathway has been proposed (Figure 1: Proposed metabolic pathway of chlorophene in rats).

Figure 1: Proposed metabolic pathway of chlorophene in rats

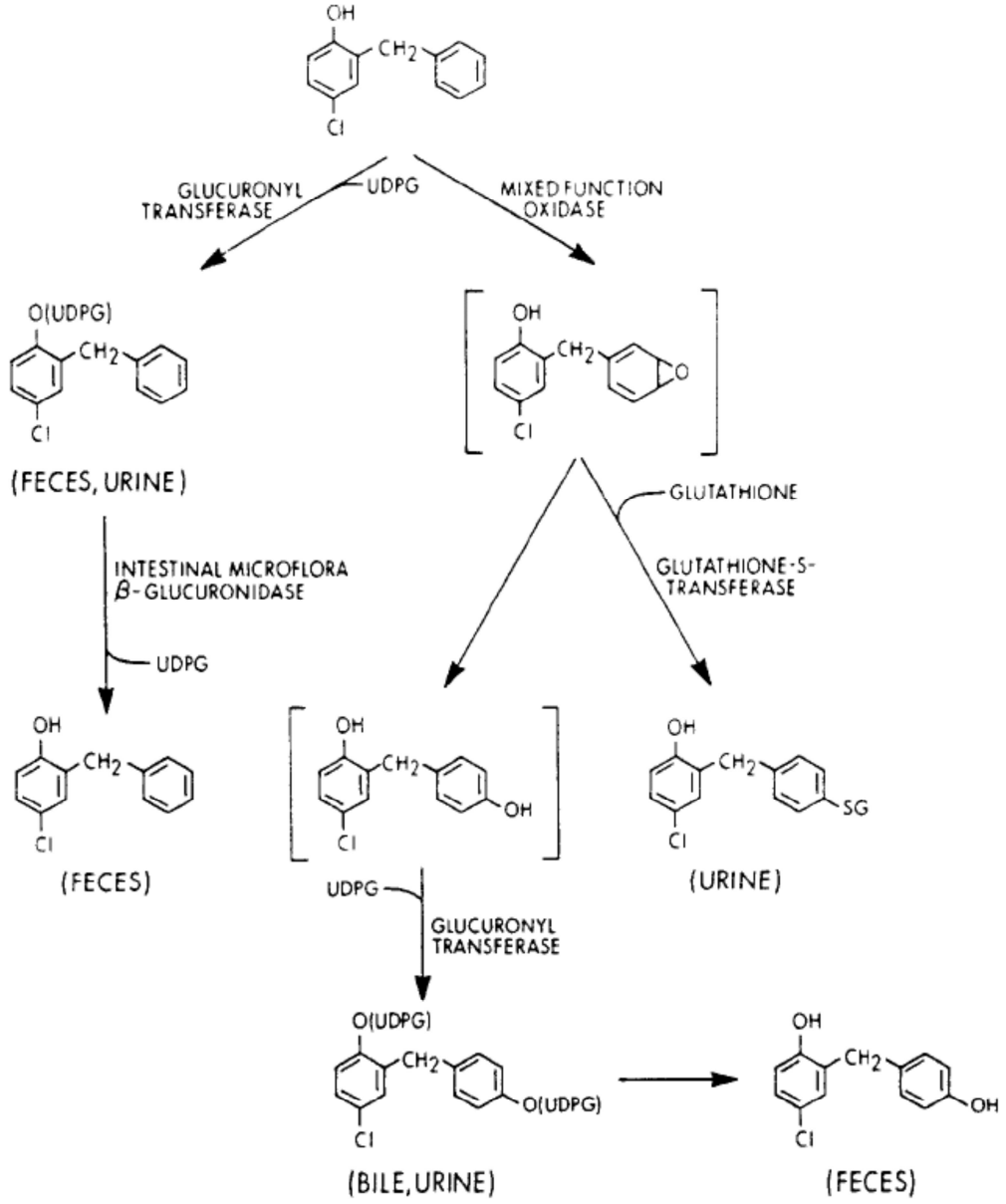


Table 8: Distribution of chlorophene in tissues after different routes of exposure[a] *(figures from Doc IIIA; Table A6_2-2)*

Percent of total dose		
Tissue	**Intravenous**	**Oral**
	10 mg/kg	**10 mg/kg**
All tissues	**1.31**	**0.68**
Urine	34.9	24.5
Faeces	53.78	76.3

Recovery[b]	**89.98**	**101.44**
[a] at 72 h post administration [b] radioactivity in all tissues +excreta		

Dermal absorption of chlorophene from a commercial 5% disinfectant solution was determined *in vivo* in four male rats (Sprague-Dawley) per group (confidential, 1994 / A6_2(2)). The doses used were 10, 100 and 1000 mg of formulation/ml which corresponds to 0.5, 5 or 50 mg ^{14}C chlorophene/ml. The solution was diluted in water and applied on a 15 cm^2 area of shorn and intact dorsal skin. The exposure periods were 1, 4, and 10 h. Before termination the protective cover was removed and the treated skin was wiped with gauze pads. Levels of ^{14}C chlorophene were determined by liquid scintillation counting. An additional group was treated for 10 h and sacrificed after 168 h to assess the dynamics of excretion and observation of clinical signs. One animal from the mid-dose group showed alopecia on day 7, while an animal from the high-dose group showed increased reactivity on post-treatment days 3 through 5. Since these effects started late during the observation period they were not considered to be treatment related. Absorption of chlorophene was incomplete over the course of a 10 h exposure. The absorption values for all three dose groups sacrificed after 10 h ranged from 41% to 52%, while the values ranged from 42% to 60% for the group sacrificed after 168 h. The dose was calculated based on the levels measured in skin, blood, urine, faeces, remaining carcase and cage wash (Doc IIIA; Table 6 2-2: Distribution of label in the analysed compartments (10-h exposure) [% of applied dose]). Between 3% and 15.5% of the dose remained in the skin after 168 h. About half of the absorbed material was absorbed during the first hour. The lack of strong differences between dose groups indicates that no concentration-dependent limit for the rate of chlorophene absorption exists in the tested dose range. After 10 h the majority of the radiolabelled material was found in the urine, whereas for the animals sacrificed after 168 h a higher portion of labelled material was found in the faeces. Based on the highest measured dermal absorption value in this study the dermal absorption for chlorophene was 60%.

4.1.1 Summary and discussion of toxicokinetics

In an ADME study of chlorophene in rats oral administration of chlorophene resulted in higher relative percentages of chlorophene excreted in the faeces compared compared to i.v. administration. After dermal application, a high percentage of the total dose of chlorophene was present at the application site. These findings indicated that chlorophene was incompletely absorbed through both intestine and skin.

Since the levels in bile were not measured after oral administration in the submitted ADME study, an oral absorption could be estimated based on the lowest urine excretion in addition to the chlorophene levels found in the tissues. As this assumption is assumed to be too conservative; an estimation of oral absorption for chlorphene based on results from an oral and IV administration of test substance (measurement of net test substance present in urine plus expired air plus carcass by each of the two routes) was used. Based on these assupmtions an **oral absorption of 70 %** was decided for chlorophene. Based on the levels in urine, faeces and tissues, **dermal absorption of**

chlorophene was approximately **62%** in the study of Kao & Birnbaum while in the other study (confidential, 1994 / A6_2(2)) the dermal absorption value was approximately **60%.**

Most of the administered chlorophene was excreted and the tissue levels were generally low within 3d post administration (except for the dermal study where 32 % of the total dose was found at the skin site). However, the highest concentration of BCP-derived radioactivity was found in the kidney during the whole measuring period. This affinity of renal tissue for chlorophene is likely to play a role in the suggested nephrotoxicity of this compound. In addition, the studies indicated that enterohepatic circulation was involved in chlorophene disposition.
The major *in vivo* metabolites detected after BCP exposure were glucuronyl conjugates of chlorophene and hydroxy-chlorophene in faeces and urine.

4.2 Acute toxicity

Summary table of relevant acute toxicity studies:

Table 9: Acute toxicity of chlorophene

Route	**Method Guideline**	**Species, Strain, Sex, No/group**	**Dosage**	**Value LD_{50}/LC_{50}**	**Reference**
Oral	LD_{50} test ≅ OECD 401 Non-GLP	Rat CD, Sprague-Dawley derived ♂+♀, 5/sex/dose	Single dose at 1500, 2500, 3150, 3969, 5000 mg/kg bw. Post exposure period, 14 days	LD_{50} = 3852 mg/kg	Confidential,1983a A6_1_1 **KEY STUDY**
Dermal	Limit test ≅ OECD 402 Non-GLP	Rat CD, Sprague-Dawley derived ♂+♀, 5/sex/dose	Single dose of 2000 mg/kg bw. Exposure duration, 24 hours.	LD_{50} > 2000 mg/kg	Confidential,1983b A6_1_2 **KEY STUDY**
Inhalation	LC_{50} test ≅ OECD 403 Non-GLP	Rat CD, Sprague-Dawley derived ♂+♀, 5/sex/concen-tration	Nose-only: 2.07, 2.40, 3.13 mg/L. Duration of exposure 4 h	LC_{50} = 2.43 mg/L/4h **Acute Tox 4, H332 Harmful if inhaled**	Confidential, 1983c A6_1_3 **KEY STUDY**

4.2.1 Summary and discussion of acute toxicity

Chlorophene is of low acute toxicity by the oral (LD_{50} = 3852 mg/kg bw) and percutaneous route (LD_{50} > 2000 mg/kg bw) and of moderate toxicity via inhalation (LC_{50} = 2.43 mg/L/4h). Following inhalation increased lung weights were noted in the decedents, indicating pulmonary irritation and respiratory failure caused by oedema. Three cases of hydronephrosis, four cases of enlarged cervical lymph nodes and one case of hepatic nodule were observed in the decedents after exposure via inhalation.

4.2.2 Comparison with the CLP criteria

The criteria for classification with acute toxic by inhalation category 4 is fulfilled. The LC_{50} value of 2,43 mg/L/4h is > 1 but < 5 (dust/mist), and meets the criteria for classification in category 4.

4.2.3 Conclusions on classification and labelling

According to Regulation (EC) No 1272/2008 (CLP) chlorophene should be classified as:

Acute toxic, Category 4 H332: Harmful if inhaled

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal According to the DS, Chlorophene was of low acute toxicity by the oral and dermal routes (LD_{50} = 3852 mg/kg bw and LD_{50} > 2000 mg/kg bw, respectively) and of moderate acute toxicity via the inhalation route (LC_{50} = 2.43 mg/L/4h). The criteria for classification for acute toxicity by inhalation as Acute Tox. 4 was fulfilled (1 < LC_{50} ≤ 5 mg/L/4h for dusts and mists). **Comments received during public consultation** Four Member State Competent Authorities (MSCA) and one Industry source responded during the public consultation, all of whom agreed with the classification proposal. **Assessment and comparison with the classification criteria** RAC agrees with the DS that classification is required for acute inhalation toxicity of chlorophene. The available data show that the mean LC_{50} value for male and female Sprague-Dawley rats combined is 2.43 mg/L/4h. This finding is in accordance with the criteria for classification as Acute Tox. 4 (H332) for dusts and mists (1 < LC_{50} ≤ 5 mg/L). The LD_{50} reported in an acute oral toxicity test Sprague-Dawley rats was 3852 mg/kg for males and females combined, which is above the guidance value for classification by the oral route (Acute Tox. 4, H302: 300 < LD_{50} ≤ 2000 mg/kg). The LD_{50} reported in an acute dermal toxicity test in male and female Sprague-Dawley rats is also above the guidance vale for classification by the dermal route (Acute Tox. 4, H312: 1000 < LD_{50} ≤ 2000 mg/kg). Therefore, RAC agrees with the DS that the data support no classification for acute toxicity by the oral or dermal routes and classification of chlorophene as **Acute Tox. 4 by the Inhalation route (H332 – harmful if inhaled)**.

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

4.3.1 Summary and discussion of specific target organ toxicity – single exposure

No significant or severe toxicity to a specific organ in the absence of lethality was observed in acute oral, inhalation or dermal toxicity studies in animals. Additionally, no acute organ toxicity was observed in short-term or long-term studies.

4.3.2 Comparison with the CLP criteria

The observed effect in the relevant animal studies of chlorophene does not meet the CLP criteria for classification for specific target organ toxicity after single exposure.

4.3.3 Conclusions on classification and labelling

No classification proposed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

No significant or severe toxicity to a specific organ (in the absence of lethality) was observed in acute oral, inhalation or dermal toxicity studies in animals. In addition, there was no organ toxicity observed during the first days of dosing in short-term or long-term studies. Therefore, no classification for specific target organ toxicity after a single exposure was proposed by the DS.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Classification for specific target organ toxicity following a single exposure (STOT SE 3), is primarily based on human evidence with data from animal experiments providing support in a weight-of-evidence assessment. The criteria for classification as STOT SE 3 for respiratory tract irritation include effects on the lungs which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period of time without leaving significant alteration of structure or function. Specifically, animal studies may provide information in terms of clinical signs of toxicity and histopathology (e.g. hyperaemia, oedema, minimal inflammation, thickened mucous layer) which are reversible.

Following an acute toxicity study by the inhalation route, it was noted that decedents had increased lung weights indicative of pulmonary inflammation and respiratory failure caused by oedema. Whilst these effects might be indicative of severe respiratory tract irritation, they only occurred in decedents and there was no indication of such effects in surviving animals. As concluded by the DS, there was no other significant or severe organ toxicity in the acute toxicity studies.

Therefore, as the pulmonary effects occurred in the presence of lethality and there were no other significant or severe organ toxicity noted, RAC agrees with the DS that **no classification** for STOT SE is warranted.

4.4 Irritation

4.4.1 Skin irritation

Summary table of relevant skin irritation studies:

Table 10: Skin irritation by chlorophene

Species	Method	Average score 24, 48, 72 h		Reversibility	Result	Reference
		Erythema	Oedema			
Rabbit	OPPTS 870.2500 ≅ OECD 404	2.89	4.00	Yes	**Skin Irrit. 2 H315 Causes skin irritation**	Confidential, 2000 A6_1_4(1) **KEY STUDY**

Rabbit	≅ OECD 404 Non-GLP	1.22	0.22	Yes	Moderately irritating, exfoliation, eschar formation	Confidential, 1983d
Rabbit	No Guideline Non-GLP	4.00 (48 h only)	2.25 (48 h only)	Yes	Strongly irritating	Confidential, 1983

Table 11: Skin irritation (individual scores) of chlorophene (confidential, 2000 /A6_1_4(1))

Observation time	Rabbit no.					
	F05790		F05791		F05792	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
4 h	2b	4	2	4	2b	4
24 h	2b	4	2b	4	2b	4
48 h	3b	4	2b	4	2b	4
72 h	4n	4	4n	4	4n	4
96 h	4n	4	4n	4	4n	4
Mean value 24 + 48 + 72 h	3.0	4.0	3.0	4.0	2.7	4.0
Reversibility	Yes	Yes	Yes	Yes	Yes	Yes
Avg. time for reversion	21 d	14 d	14 d	14 d	14 d	14 d

b blanching
n necrotic appearance

4.4.1.1 Summary and discussion of skin irritation

Chlorophene caused strong irritation on the skin of rabbits with strong erythema and oedema. The key study was performed according tof OECD guideline 404.

4.4.1.2 Comparison with the CLP criteria

Classification proposal according to Regulation EC 1272/2008: Skin irritant category 2

The criteria for classification with skin irritation category 2 is fulfilled;

Mean value of ≥ 2,3 - ≤ 4,0 for erythema/ eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions;

4.4.1.3 Conclusions on classification and labelling

Chlorophene was tested on the skin of rabbits. It caused strong irritation on the skin with strong erythema and oedema. The overall results fulfil the criteria of Directive 2001/59/EC and Regulation (EC) No 1272/2008 (CLP) for classification as a skin irritant:

Skin Irrit Cat 2. H315: Causes skin irritation

4.4.2 Eye irritation

Summary table of relevant eye irritation studies:

Table 12: Eye irritation of chlorophene

Species	Method	Average score 24, 48, 72 h				Reversibility	Result	Reference
		Cornea	Iris	Conjunctiva				
				Redness	Chemosis			
Rabbit	≅ OECD 405 Non-GLP	2.78	0.89	2.67	1.78	No	**Eye Dam 1 H318 Causes serious eye damage**	Confidential, 1983e A6_1_4(2) **KEY STUDY**
Rabbit	No Guideline Non-GLP	1.50	1.33	2.17	2.00	Yes	Irritating	Confidential, 1983

Table 13: Eye irritation of chlorophene (confidential, 1983e / A6_1_4(2))

	Cornea	Iris	Conjunctiva	
			Redness	Chemosis
Score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to 4
1 h	1.67	0.00	1.00	2.00
24 h	2.33	0.67	2.00	1.67
48 h	3.00	1.00	3.00	1.67
72 h	3.00	1.00	3.00	2.00
Average 24 h, 48 h, 72 h	**2.78**	**0.89**	**2.67**	**1.78**

Table 14: Eye irritation, individual data of chlorophene (confidential, 1983e / A6_1_4(2))

Observation time	Rabbit nr SP 353 M			
			Conjunctiva	
	Cornea	Iris	Redness	Chemosis
1 h	1	0	1	2
24 h	2	1	2	2
48 h	3	1	3	2
72	3	1	3	2

Observation time	Rabbit nr SP 354 M			
			Conjunctiva	
	Cornea	Iris	Redness	Chemosis
1 h	2	0	1	2
24 h	3	0	2	2
48 h	3	1	3	2
72	3	1	3	2

Observation time	Rabbit nr SP 356 M			
			Conjunctiva	
	Cornea	Iris	Redness	Chemosis
1 h	2	0	1	2
24 h	2	1	2	1
48 h	3	1	3	1
72	3	1	3	2

4.4.2.1 Summary and discussion of eye irritation

Chlorophene caused significant irritation of the eye in tests on albino rabbits (confidential, 1983e / A6_1_4(2)). Lesions of cornea and iris as well as conjunctival redness and chemosis, all of which persisted until the end of the observation period, were noted.

The study was terminated 72 h after treatment in light of the deteriorating condition of the treated eyes, especially the corneae. It was considered that significant resolution of the treatment effects was most improbable within the period of extended observation allowed by the OECD test method.

The study was performed according to OECD 405. No formal GLP compliance statement (GLP was not compulsory during the conduct of study) was applied, but there was a signed QAU statement.

4.4.2.2 Comparison with the CLP criteria

Classification proposal according to Regulation EC 1272/2008: Eye dam. Cat 1,

The criteria for classification with eye damage is fulfilled;

If, when applied to the eye of an animal, a substance produces: — at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/ or — at least in 2 of 3 tested animals, a positive

response of: — corneal opacity ≥ 3 and/or — iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

4.4.2.3 Conclusions on classification and labelling

Chlorophene produced ocular irritation characterised by diffuse opacity or translucency of the whole visible corneal surface, injection of the conjunctival blood vessels and eversion of the eyelids due to moderate chemosis. Positive irritation reactions were observed in all animals.

Irritation responses of the treated eyes and other effects of treatment became more marked at each subsequent examination and culminated in a state of severe ocular irritation four days after treatment. The study was terminated 72 h after treatment due to animal welfare and reversibility was not expected.

Chlorophene should be classified as:

Eye dam. Cat1, H318: Causes serious eye damage according to Regulation (EC) No 1272/2008 (CLP).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Three studies were summarised in the CLH report. The key study was performed according to OECD Test Guideline (TG) 404. Chlorophene caused strong irritation on the skin of rabbits characterised by severe erythema and oedema. According to the DS, the overall results demonstrated that chlorophene fulfilled the criteria for classification as Skin Irrit. 2.

Comments received during public consultation

Four MSCA and Industry agreed with the classification proposal. One MSCA requested more information on the nature of the necrotic appearance of the skin observed in one of the available studies.

Assessment and comparison with the classification criteria

Two of the three available studies followed OECD TG 404 without major deviations. The most recent study (dated 2000) showed that chlorophene caused mean erythema and oedema scores of 2.89 and 4.00 respectively (24, 48 and 72 h) in all three rabbits. The effects observed were reversible within 14 days for 2/3 rabbits and within 21 days for 1/3 rabbits. At 72 and 96 h, the study authors noted that the erythema had a necrotic appearance in all three rabbits During the public consultation the DS detailed observations at timepoints beyond 96h (see "Supplemental information - In depth analyses by RAC" in the background document (BD); Annex 2). At 14 and 21 days all animals were described as having "scar-like tissue".

However, a clear corrosive response indicating visible necrosis through the epidermis and into the dermis was not described in any animal following exposure to chlorophene. Therefore, the results of this study are considered consistent with the classification criteria for skin irrit. 2 since both mean erythema and oedema scores were above 2.3, but not greater than 4.0.

The second study (dated 1983), which followed a method similar to OECD TG 404, non-GLP, observed average scores for erythema and oedema of 1.22 and 0.22 respectively. Chlorophene caused exfoliation and eschar formation but these effects were considered reversible. The result of the study showed that chlorophene was moderately irritating to the skin but the mean scores did not fulfil the criteria for classification for skin irritation.

The third study provided (also dated 1983) was not carried out according to OECD TG 404 or GLP. Irritancy scoring for erythema and oedema was only given for the 48 h timepoint, with scores of 4 and 2.25, respectively. Chlorophene was determined to be strongly irritating in this study but effects observed were considered reversible. No classification can be determined from this study as there was no scoring given for the 24 or 72 h timepoints.

Additional information on the skin irritant potential of chlorophene can be taken from the skin sensitisation studies (see below). Briefly, the results of several Buehler tests indicated that the irritancy of chlorophene to the skin of Guinea pigs was influenced greatly by the solvent used for application. However, in one study, moderate to strong erythema reactions were observed at the sites treated with 10 % w/v chlorophene mixture during the induction phase. This supports the RAC opinion that chlorophene should be classified as a skin irritant.

RAC concludes that the criteria for classification are fulfilled in at least 2/3 tested animals. The mean values for erythema or oedema were within the guidance range of ≥2.3 - ≤ 4.0 from grading at 24, 48 and 72 h after patch removal.
RAC also concludes that classification as Skin Corr. 1 is not justified on the basis that the damage seen in skin tests with rabbits did not show clear, reproducible signs of corrosivity and was found to be reversible within 21 days.

Therefore, RAC agrees with the DS that the data support the classification of chlorophene as Skin Irrit. 2 (H315).

Supplemental information - In depth analyses by RAC

The table below provides a summary of individual skin irritation scores, compiled by RAC using additional information provided by the DS after public consultation.

Table. Summary of individual skin irritation scores in rabbits from one study (2000)

	Individual Scores for 3 rabbits	
Observation time	**Erythema**	**Oedema**
24 h	2b, 2b, 2b	4, 4, 4
48 h	3b, 2b, 2b	4, 4, 4
72 h	4n, 4n, 4n	4, 4, 4
96 h	4n, 4n, 4n	4, 4, 4
7 d	4x, 4x, 4e	3, 2, 4
14 d	1d,s, 0s, 0s	0, 0, 0
21 d	0s, 0s, 0s	0, 0, 0
Mean (24 – 72 h)	3, 2.7, 2.7	4, 4, 4
Reversibility	Yes - within 21 days	Yes - within 14 days

b = blanching
d = desquamation
e= eschar
n = necrotic appearing area
s = scar-like tissue
x = exfoliation

4.5 Sensitisation

4.5.1 Skin sensitisation

Summary table of relevant skin sensitisation studies:

Table 15: Skin sensitisation data for chlorophene

Species	Method	Number of animals sensitised/ total number of animals	Result	Reference
Guinea pig	Buehler Test OPPTS 870.2600 ≅ OECD 406	24 h: 15/20 48 h: 18/20 72 h: 19/20	**Skin sensitising Cat 1A** **H317 May cause an allergic reaction**	Confidential, 2001 A6_1_5 **KEY STUDY**
Guinea pig	Buehler Test OECD 406	9/20	Sensitising	Confidential, 2002
Guinea pig	Klecak Test No guideline	0/24	Not sensitising	Confidential, 1986

4.5.1.1 Summary and discussion of skin sensitisation

Chlorophene was tested for its skin sensitisation potential in Buehler and Klecak tests on Guinea pigs. The first Buehler test (confidential, 2001 / A6_1_5) used 10% and 5% solutions (10 % was the induction dose) of chlorophene in propylene glycol whereas the second Buehler study (confidential, 2002) featured induction and challenge concentrations of both 50% in polyethylene glycol 400.

In the Buehler test by Glaza moderate to strong erythema reactions were observed at the sites treated with the 10% w/v mixture during the induction phase. Subcutaneous haemorrhaging, blanching, and/or necrotic appearing areas were also observed within the induction sites of all test animals.

After the challenge with the 5% test substance mixture, 19 out of 20 of the test compound group showed very faint to moderate redness. Subcutaneous haemorrhaging, desquamation or fissuring was also observed within the challenge sites of 3 test animals. Very faint to faint erythema was observed in 2 of the 10 naïve control animals after challenge.

4.5.1.2 Comparison with the CLP criteria

According to the criteria mentioned in the Regulation No 1272/2008 on CLP' *(> 60 % responding at > 0,2 % to 20 % induction dose*); the substance should be classified for skin sensitisation, category 1A: H317 May cause an allergic skin reaction (strong potency on basis of the Buehler Occluded Patch Test).

4.5.1.3 Conclusions on classification and labelling

Based on the positive outcome of two Buehler tests, chlorophene should be classified as:

Skin sensitisation, category 1A with H317: May cause an allergic skin reaction according to Regulation (EC) No 1272/2008 (CLP)**.**

4.5.2 Respiratory tract sensitisation

4.5.2.1 Non-human information:

No information available.

4.5.2.2 Human information:

No information available.

4.5.2.3 Conclusions on classification and labelling

There are no human or animal data indicating sensitization following inhalation, thus classification is not warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The DS summarised three studies in the CLH report. Chlorophene was tested for its skin sensitisation potential in two Buehler tests (dated 2001 and 2002, respectively) and one open epicutaneous test (Klecak test) on guinea pigs (dated 1986). The classification proposal was based on positive outcomes from the two Buehler tests. In the 2001 study, after induction using 10% chlorophene solution, a challenge dose of 5% chlorophene solution was applied to the animals. Out of 20 animals tested, 19 showed faint to moderate redness. Classification with Skin Sensitisation Category 1A (Skin Sens. 1A; H334) was proposed by the DS on the basis of ≥ 60% of animals responding at > 0.2% to ≤ 20% induction dose.

Comments received during public consultation

Four MSCA commented on the proposed classification: three supported Skin Sens. 1A (H317); one requested more information and expressed doubts about the proposal. One of the MSCAs supporting the proposal commented that the observation of ≥ 60% respondents in the Buehler test dated 2001 following induction at a topical concentration between 0.2 and 20% supported their position due to the poor and possibly unreliable study. Specifically, the 10 % concentration of chlorophene used at induction was too high as it caused moderate to strong erythema during induction and also subcutaneous haemorrhaging, blanching and necrotic appearing areas in test animals. This MSCA pointed out that the Buehler test guideline recommends that the concentration of the test substance at induction should be the highest to cause mild irritation. This MSCA requested more details of the 2002 Buehler test, in which it had apparently been reported that 45% of test animals (9/20) had skin reactions when tested with 50% chlorophene at induction and challenge, suggesting that a classification as Skin Sens. 1B might be more appropriate.

The manufacturer of chlorophene proposed Skin Sens. 1B; H317. In support of this, they provided a critical assessment of the two Buehler tests and the open epicutaneous test summarised in the CLH report. They also provided details of a third Buehler test on chlorophene (dated 2005), two further Buehler tests on disinfectant formulations that

included chlorophene, a ranking of chlorophene as a skin sensitiser made by the German Federal Institute for Risk Assessment, and a brief summary of several human studies. The additional data are summarised in the BD (Annex 2).

Although the DS initially proposed the classification Skin Sens 1A, based on a response rate in a Buehler test of >60% at > 0.2 to 20% induction dose, this position changed in light of the comments and additional data received during the public consultation. In response to the public consultation, the DS commented that the classification Skin Sens 1 (H317) now seemed the most appropriate, i.e. without any sub-categorisation. They agreed that the results of the two Buehler tests included in the CLH report had to be interpreted with care as the dose selection at induction and challenge had been inappropriately high.

Additional key elements

During public consultation, further useful information was provided by the manufacturer of chlorophene. The data are summarised below.

1. Animal data

Buehler test (2001): solvent used was propylene glycol; the concentrations used for induction (10%) were too high and caused moderate to strong irritation with necrotic-appearing areas of skin. The third induction consequently was performed at a different site, although strong erythema also occurred possibly as a consequence of arising excited skin syndrome, also termed angry skin syndrome (Anderson and Maibach, 1980[1]), a symptom of nonspecific hypersensitivity related to the experimental conditions. Industry argued that the position on classification should not be based on this study alone, but on a weight of evidence taking into account all available studies.

Buehler test (2002): solvent was polyethylene glycol 400, resulting in a lower irritant potential of the test substance such that 50% chlorophene was used for both induction and challenge. Slight irritation was observed after the third induction in 7/20 animals. At challenge, slight to moderate effects were induced in 45 % of animals (9/20). The results of this study would fall into category 1B for classification (≥ 15 % of animals responding to > 20 % topical induction dose).

Buehler test (2005): this test was not included in the CLH report. Chlorophene was applied in a concentration of 0.5% in ethanol/water (80/20) for induction. For challenge, a concentration of 0.25 % in acetone was used. After challenge, faint erythema was observed (score 0.5) in 4/10 animals and also 2/10 control animals. Strictly, the results of this study place chlorophene in category 1B (≥ 15% to < 60% responding to >0.2% to ≤ 20% topical induction dose). Industry also commented that the doses used in this study might have been too low to accurately detect skin sensitisation.

Open epicutaneous test (Klecak test) (1986): guinea pigs were treated with 1, 3 or 10 % chlorophene in propylene glycol. After the first 5 days of treatment the concentration of the 10 % group was reduced to 3 % and the treatment area was changed due to strong cumulative skin effects (similar to observations in the Buehler 2001 study: erythema, oedema and encrustation in all animals). After challenge with 0.3%, 1% and 3% chlorophene, no skin sensitising effects were observed. Therefore, chlorophene would not be classified for skin sensitisation on the basis of this study.

Two further Buehler tests (1998): guinea pigs treated with two disinfectant formulations containing concentrations of 1% and 0.2% chlorophene, respectively. The results were negative. Industry concluded that whilst these results did not provide a case for a lack of skin sensitisation of chlorophene, they might add to the weight of evidence that the

substance is not a strong sensitiser.

2. Human data (none of which was included in the CLH report; references cited in full by industry)

Dohn (1980): 1 of 221 patients reacted positively to 25% chlorophene in water.

Rothe *et al* (1993): 7 of 371 people with a suspected contact dermatitis towards disinfectants reacted positively to pure chlorophene.

Sonnex and Rycroft (1986): case study of a person who reacted positively to 1% chlorophene, whereas of the 50 control subjects 47 did not show any reaction to chlorophene and 3 showed mild irritant reactions only.

Kahn *et al*. (1970): 3 of 13 people reacted positively to chlorophene as well as to two other
phenolic constituents tested, indicating that cross-hyperactivity can occur

3. Assessments by other bodies

Schlede *et al*. (2003): In a potency ranking of 244 substances performed by an expert group on skin sensitisation at the German Federal Institute for Risk Assessment (BfR), chlorophene was judged as a substance with 'insignificant or questionable allergenic effect' (Category C).

Assessment and comparison with the classification criteria

RAC is of the opinion that the weight of evidence is sufficient to justify classification of chlorophene as a sensitiser. The available data are summarised in the table below .

Table. Summary of skin sensitisation data

Test (date)	Result	Observations and conclusions
Buehler (2001)	Positive	Induction and challenge doses gave a significant irritant response. Although > 60 % of animals were described as sensitised at 24, 48 and 72 h following induction with 10% chlorophene, it is unclear how this was influenced by the irritant nature of the treatment. Potency cannot be assessed reliably from this study.
Buehler (2002)	Positive	A response rate of 45% (9/20) was seen. With a different solvent employed to that in the 2001 study, chlorophene was less irritating and a 50% concentration was used at induction and challenge. The result suggests moderate potency (>= 15% sensitised at > 20% induction concentration), but a higher potency cannot be excluded from this result.
Buehler (2005)	Positive	Only 10 animals per dose group were used. After challenge, very faint erythema was seen in 4/10 and 2/10 treated and control animals, respectively. This is a positive result (20% response rate), but not sufficient to indicate high potency

		(>60% response rate). The induction dose of 0.5% led only to very faint desquamation in ¼ animals in a dose-range finding study, whereas 1% produced a response in all animals. It cannot be discounted that a 1% induction concentration would have produced a more potent response; higher potency cannot be excluded from this result.
Klecak (1986)	Negative	Not a guideline study. The negative result at least adds support to the view that chlorophene is not a potent skin sensitiser.
Human data from clinical tests in people already sensitised	Positive	The available information from clinical tests shows that chlorophene has potential to elicit skin sensitisation reactions in people. However, the information is limited and does not include any useful quantitative information on induction exposure or potency.

The 3 positive Buehler tests each have shortcomings, but collectively they provide a sufficient basis for classification of chlorophene as a skin sensitiser. However, the data are insufficient to justify classification of chlorophene as a potent skin sensitiser. The original proposal was based on the results of the Buehler test conducted in 2001 and it is now clear that the test concentrations used in the induction and challenge phases of this study were too high. Accordingly, the study cannot be used to provide a reliable estimate of potency. Similarly, the results do not provide an unequivocal profile of moderate potency - as discussed in the Guidance on the Application of the CLP Criteria (version 4: November 2013), sufficient information is not available to exclude the possibility of chlorophene being a strong sensitiser.

RAC is of the opinion that chlorophene should be classified as Skin Sens. 1 (H317: May cause an allergic skin reaction).

4.6 Repeated dose toxicity

Summary table of relevant repeated dose toxicity studies:

Table 16: Subacute, subchronic, and chronic toxicity of chlorophene

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
Oral, gavage	16 days ≅ OECD 407 Non-GLP	Rat F344 ♂ + ♀ 5/sex/group	0, 62.5, 125, 250, 500, 1000 mg/kg	Absolute and relative kidney weights were significantly increased in males at ≥ 125 mg/kg bw/day and in females	LO(A)EL = 125 mg/kg bw/ day NO(A)EL = 62.5 mg/kg bw/	Sendelbach, 1982a A6_3_1 **KEY**

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
			bw/day Once daily, 5 days/week	at 500 and 1000 mg/kg bw/day. Absolute and relative liver weights were significantly increased in males at ≥ 250 mg/kg bw/day and in females at 1000 mg/kg bw/day. At ≥ 250 mg/kg bw/day: absolute and relative thymus weight was decreased in females. At ≥ 500 mg/kg bw/day: absolute and relative thymus weight was decreased in males. Mild to moderate nephropathy was observed in all animals of the 1000 mg/kg bw/day group. The incidence and severity increased with dose (incidence: 62.5 mg/kg 1/10; 125 mg/kg 2/10; 250 mg/kg 2/10; 500 mg/kg 7/10). Myocardial degradation was observed in 8/10 rats of the 1000 mg/kg bw/day group. Two females in the 1000 mg/kg bw/day-group died during the study.	day	**STUDY**
Oral, gavage	16 days ≅ OECD 407 Non-GLP	Mouse $B6C3F_1$ ♂ + ♀ 5/sex/group	0, 62.5, 125, 250, 500, 1000 mg/kg bw/day Once daily, 5 days/week	Absolute and relative liver weights were increased at ≥ 250 mg/kg bw/day in females and at 500 mg/kg bw/day in male mice. At 500 and 1000 mg/kg bw/day nephropathy was observed in some mice. No changes in relative kidney weight were reported. At 1000 mg/kg bw/day: 3 males and 5 females died.	LO(A)EL = 250 mg/kg bw/ day NO(A)EL = 125 mg/kg bw/ day	Sendelbach, 1982b
Oral, capsule	21 days No guideline Non-GLP	Dog Beagle ♂+♀ 3/sex/group. In 100 mg/kg bw/day group: 5 males, 1 female	0, 3, 10, 30, 100 mg/kg bw/day Once daily, 7 days/week	At 100 mg/kg bw/day; lower overall body weight gain. Two of three male dogs at 30 mg/kg bw/day and one of five at 100 mg/kg bw/ day died during the study	LO(A)EL = 100 mg/kg bw/ day NO(A)EL = 30 mg/kg bw/ day	Confidential, 1973a
Dermal	5 day dose-finding study No	Rabbit NZW ♂+♀ 1/sex/group	2.4, 60, 125, 500 mg/kg bw/day. Once	No systemic effects. Local reactions comprised erythema, oedema, atonia and discolouration of the skin.	Systemic effects: NO(A)EL = 500 mg/kg bw/ day	Confidential, 1984

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
	guideline		daily, 6 h/day, 5 days. Group 2.4 mg/kg bw/day and 60 mg/kg bw/day animals sacrificed at day 6 and the remaining animals sacrificed at day 8	Slight to moderate skin effects (erythema) at 1% and 2,5% Chlorophene in 50% v/v ethanol in distilled water. Moderate to severe skin effects (erythema, atonia, oedema) was observed at ≥ 5%.	(highest dose) Local effects: LO(A)EL = 1 % NO(A)EL = 2,5% Chlorophene in 50% v/v ethanol in distilled water	
Dermal	4 weeks US-EPA 82-2 ≅ OECD 410	Rabbit NZW ♂+♀ 7/sex/group	1, 5, 25 mg/ kg bw/day Once daily, 6 h/day 7 days/week	No systemic effects. At 25 mg/kg bw/day local skin effects were observed (erythema, atonia, discoloration). Occasional animals treated with 5 mg/kg bw/day displayed slight to moderate skin effects	Systemic effects: NO(A)EL = 25 mg/kg bw/ day (highest dose) Local effects: LO(A)EL = 5 mg/kg bw/day NO(A)EL = 1 mg/kg bw/day	Confidential, 1989
Dermal	3 weeks US-EPA 8.22 ≅ OECD 410	Rabbit NZW ♂+♀ 7/sex/group	0, 4, 20, 100 mg/kg bw/day Once daily, 6 h/day 7 days/week	A total of 14 animals died during the treatment period (0 mg/kg 2 (♂)/14; 4 mg/kg 2(♂)/14; 20 mg/kg 5(3♂+2♀)/14; 100 mg/kg 5(3♂+2♀)/14). Kidney and liver lesions were rather more common among decedents which had been treated with chlorophene. A higher number of female rabbits had kidney lesions at the time of death (dead/killed during treatment period + sacrificed at the end of the study) in the highest dose group, and there were a tendency towards more severe kidney lesions in the 100 mg/kg bw/day group compared to the control group. At 100 mg/kg bw/day Alkaline phosphatase (ALP) was significantly decreased in	Systemic effects based on kidney lesions: LO(A)EL= 100 mg/kg bw/day NO(A)EL = 20 mg/kg bw/ day Local effects: LO(A)EL = 20 mg/kg bw/day NO(A)EL = 4 mg/kg bw/day	Confidential, 1985 A6_3_2(1) **KEY STUDY**

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
				females. Local reactions to treatment with chlorophene were observed at ≥ 20 mg/kg bw/day.		
Dermal	3 weeks ≅ OECD 410	Rabbit HC:NZW ♂+♀ 5/sex/group	0, 10, 40, 160 mg/kg bw/day Once daily, 6 h/day 5 days/week	A dose-dependent increase in histopathological changes in the kidneys was observed in females treated with ≥ 40 mg/kg bw/day. The changes included tubular calcinosis, tubular proliferation and cellular infiltration. Absolute and relative liver weights were significantly decreased in females at 160 mg/kg bw/day. At 160 mg/kg ALP was reduced in males and females. Local reactions to treatment were observed in all animals treated with ≥ 40 mg/kg bw/day. One male control + two females (one control and one low-dose) were killed in extremis.	Systemic effects: LO(A)EL = 40 mg/ kg bw/day NO(A)EL = 10 mg/kg/ day **STOT RE1** **H372: Causes damage to kidneys through prolonged or repeated exposure** Local effects: LO(A)EL = 40 mg/kg bw/day NO(A)EL = 10 mg/kg bw/day	Confidential, 1985 A6_3_2(2) **KEY STUDY**
Oral, gavage	95 days ≅ OECD 408	Rat F344/N 10/sex/group	0, 30, 60, 120, 240, 480 mg/kg bw/ day Once daily, 5 days/week	Increased absolute and relative kidney weights and reduced absolute and relative thymus weights at ≥ 240 mg/kg bw/day in females and at 480 mg/kg bw/day in males and females. There seems to be a dose-related increase in incidence and severity of nephropathy in male rats at ≥ 30 mg/kg bw/day, although the severity of the nephropathy was minimal to mild at these doses. At higher doses the severity of nephropathy increased (mild to moderate grade) in the 240 mg/kg bw males and in the 480 mg/kg bw males and females. The incidence of nephropathy was significantly increased in male rats at ≥ 120 mg/kg bw/day as stated in Birnbaum et al., 1986 (in the Discussion).	LO(A)EL = 120 mg/kg bw/day based on dose-related significantly increased incidence of nephropathy and dose-related increased severity of nephropathy NO(A)EL = 60	National Toxicology Program Technical Report Series No. 424 (1994) 6_4_1(1) and Birnbaum et al., 1986 **KEY STUDY**

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
				These findings could be interpreted to be of limited significance since Fischer 344 male rats spontaneously develop nephropathy (Dixon et al., 1995). However, very few of the concurrent vehicle controls animals in the current study did develop nephropathy (1/10 males) during the study. Historical control data supporting an assumption for non-significance are not provided.		
Oral, gavage	95 days ≅ OECD 408	Mouse B6C3F$_1$ 10/sex/group	0, 30, 60, 120, 240, 480 mg/kg bw/ day Once daily, 5 days/week	No effects on final body weight or body weight gain were observed. No treatment-related clinical findings or gross lesions.	NO(A)EL = 480 mg/kg bw/day (highest dose)	National Toxicology Program Technical Report Series No. 424 (1994) A6_4_1(1)
Oral, gavage	95 days ≅ OECD 408	Mouse B6C3F$_1$ 10/sex/group	0, 500, 650, 800, 1000 mg/kg bw/ day Once daily, 5 days/week	Survival decreased with increasing doses from 500 mg/kg bw/day in females and from 650 mg/kg bw/day in males. Decreased body weight gain at ≥ 500 mg/kg bw/day in males. Absolute and relative kidney weights were reduced at ≥ 500 mg/kg bw/day in males. Absolute and relative liver weights were significantly increased in females at ≥ 500 mg/kg bw/day and in males at 800 mg/kg bw/day.	LO(A)EL = 500 mg/kg bw/day (lowest dose) NO(A)EL = NA	National Toxicology Program Technical Report Series No. 424 (1994) A6_4_1(1)
Oral, capsule	90 days ≅ OECD 409 Non-GLP	Dog Beagle ♂+♀ 4/sex	0, 10, 30, 100, (200) mg/kg bw/day Once daily, 7 days/week	At 200 mg/kg bw/day: body weight was decreased (group was discontinued after 14 days). Reduced body weight gain was observed at 100 mg/kg/day. Absolute kidney weights were significantly increased in males at 100 mg/kg bw/day. Relative kidney weights were significantly increased in males at ≥ 30 mg/kg bw/day and in females at 100 mg/kg bw/day. Relative liver weights were significantly increased in males at ≥ 10 mg/kg bw/day	LO(A)EL = 30 mg/kg bw/ day NO(A)EL = 10 mg/kg bw/ day	Confidential, 1973b A6_4_1(2) **KEY STUDY**

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
				and in females at ≥ 30 mg/kg bw/day. Urinalysis showed that specific gravity was significantly reduced at 100 mg/kg bw/ day in both sexes after 90 days.		
Oral, diet	16-week dose-finding study No guideline Non-GLP	Rat ♂ 5/group	0, 150, 500, 1500, 3000 ppm *ad libitum* ≅ 0, 10.6, 39.4, 115.4, 254.4 mg/ kg bw/day	At 3000 ppm, i.e. 254.4 mg/kg bw/day: body weight ↓	LO(A)EL = 254.4 mg/kg bw/ day NO(A)EL =115 mg/kg bw/ day	Confidential, 2005
Oral, gavage	13 weeks 65 weeks 2 years US-EPA 83-2 ≅ OECD 453	Rat F344/N ♂+♀ 50/sex 3- and 15-month interim sacrifice: 10/sex each	0, 30, 60, 120 mg/kg bw/day (♂) 0, 60, 120, 240 mg/kg bw/day (♀) Once daily, 5 days/week	Severe, time- and dose-related nephropathy was observed, occurring as early as after 3 months (females, not possible to evaluate the males due to high spontaneous incidence of nephropathy). The severity of the nephropathy was significantly increased in a time- and dose-dependent manner both in males and females, with males as the most sensitive sex. The severity of nephropathy was significantly increased at 30 mg/kg (males) and 120 mg/kg (females) at 65 and 104 weeks. In male rats dosed for 104 weeks secondary hyperparathyroidism developed. Both males and females showed time- and dose-dependent increases in their relative kidney weights, with males as the most sensitive sex (♂ 30 mg/kg bw/day, ♀ 120 mg/kg bw/day, at 2 years). Similarly, time- and dose-dependent increases in heart weight were also observed (♂ 30 mg/kg bw/day, ♀ 120 mg/kg bw/day, at 2 years).	LO(A)EL= 30 / 120 mg/kg bw/day (♂/♀) based on severity of nephropathy and increased kidney weight NO(A)EL= NA* / 60 mg/kg bw/day (♂/♀) based on severity of nephropathy and increased kidney weight *Not applicable due to effect on lowest tested dose	National Toxicology Program Technical Report Series No.424 (1994) A6_5+6_7 **KEY STUDY**
Oral, gavage	Two-generation study OECD 416	Rat Wistar (HsdCpb: WU conventionally bred) 30/	0, 60, 180, 540 mg/kg bw/day ♂: 10 weeks pre-mating, 2	A significant and dose-related decrease in terminal body weight was observed in P males at ≥ 180 mg/kg bw/day and in F1 males at ≥ 60 mg/kg bw/day. Reduction in body weight gain during gestation was observed	LO(A)EL: 60 mg/kg bw in males (kidney effects) NO(A)EL: NA due to effect at lowest dose	Confidential, 2008 A6_8_2(3) (KEY STUDY for 3.8 Reproductive

Route	Duration of study, Method, Guideline	Species, Strain, Sex, no/group	Dose levels, Frequency of application	Results	LO(A)EL NO(A)EL	Reference
		sex/group	wks mating period ♀: 10 weeks pre-mating, through-out gestation and lactation	in dams at 540 mg/kg bw/day in the P generation and at 180 and 540 mg/kg bw/day in the F1 generation. Treatment-related kidney effects (nephropathy, dilated tubules, basophilic tubules and lymphocytic infiltration) were observed in P and F1 males at ≥ 60 mg/kg bw/day and P and F1 females at 540 mg/kg bw/day.		toxicity*)
Oral gavage	13 weeks 65 weeks 2 years. US-EPA 83-2 ≅ OECD 451	Mouse B6C3F$_1$ ♂ + ♀ 50/sex 3-month and 15-month interim sacrifice: 10/sex	0, 120, 240, 480 mg/kg bw/day Once daily, 5 days/week	At 2 years the absolute kidney weights of high-dose males were lower than those of the controls at the 3-month interim evaluation. Absolute and relative kidney weights of dosed male mice were lower than those of the controls, and absolute and relative kidney weights of female mice were lower than those of the controls at the mid- and high-dose levels, Kidney weights of all dosed males and females of the mid- and high dose group remained significantly reduced at the 2-year sacrifice. The severity of nephropathy was significantly increased in a time- and dose-dependent manner both in males and females, starting at the lowest dose (120 mg/kg bw), with males as the most sensitive sex. The final mean body weights of all dosed males and mid- and high dose females were lower than those of the controls.	LO(A)EL : 120 mg/kg bw/day based on increased kidney weights, and significantly time- and dose-dependent increased incidence and severity of nephropathy in both sexes. NO(A)EL: NA due to effects at lowest dose	National Toxicology Program Technical Report Series No.424 (1994) A6_7 (1) (KEY STUDY for 3.7 Carcinogenicity*)

*The studies are key studies for other chapters (3.8 Reproductive toxicity and 3.7 Carcinogenicity) and are included in the Tables in the corresponding chapters.

In rats increased kidney weights were observed in a 16-day gavage study (Sendelbach, 1982a, key study, non-GLP, doses: 0; 62.5; 125; 250; 500; 1000 mg/kg bw/day) at ≥ 125 mg/kg bw/day in males and in females at 500 and 1000 mg/kg bw/day. Mild to moderate nephropathy was observed in all animals of the 1000 mg/kg bw/day group. The incidence and severity of the nephropathy increased with dose (incidence: 62.5 mg/kg bw/day 1/10; 125 mg/kg bw/day 2/10; 250 mg/kg bw/day 2/10; 500 mg/kg bw/day 7/10). Information on the ratios between male and female rats with

nephropathy in the different dose-groups is not presented in the report from the US National Toxicology Program.

In **mice**, liver weights were significantly increased at ≥ 250 mg/kg bw/day in females and at 500 mg/kg bw/day in male mice in an analogous subacute 16-day oral gavage study (Sendelbach, 1982b, non-GLP, doses: 0; 62.5; 125; 250; 500; 1000 mg/kg bw/day). Nephropathy characterized by multifocal tubule dilatation and flattening of the proximal convoluted tubule epithelium, tubule regeneration, and minimal focal epithelial cell necrosis occurred in two 500 mg/kg bw/day and six 1000 mg/kg bw/day male and female mice.

In Beagle pups, no organ-specific effects were observed in a 21-day oral dose-range-finding study (confidential, 1973a / A6_4_1(2), doses: 0; 3; 10; 30; 100 mg/kg bw/day). Weight gain was depressed in the highest dose group (100 mg/kg bw/day). This study was performed prior to the enactment of the Good Laboratory Practice (GLP) regulations and no information on the production, purity or stability of the test material Santophen I (chlorophene) was provided in the study report.

In rabbits, three **subacute dermal studies (**all GLP) were conducted according to OECD guideline 410, with only minor deviations. In a **4-week dermal study in rabbit** (confidential, 1989, doses: 1, 5 and 25 mg/kg bw/day) no systemic effects were observed. Moderate to severe skin effects were observed at the highest dose. In addition, some animals treated with 5 mg/kg bw/day showed slight to moderate skin effects.

In a **3-week dermal study in rabbit** (confidential, 1985 /A6_3_2(1), key study; doses: 0, 4, 20, 100 mg/kg bw/day) a higher number of female rabbits of the high dose group had kidney lesions at the time of death (dead/killed during treatment period + sacrificed at the end of the study), and there was a tendency towards an increased severity of the kidney lesions in the high dose group compared to the control group (Table 17). Alkaline phosphatase (ALP) values were reduced in high-dose females. Local skin effects were observed in animals treated with 20 and 100 mg/kg bw/day of chlorophene. The skin lesions in the high dosage group were more frequent and more severe than the ones observed in the 20 mg/kg bw/day group.

Table 17: Results from repeated dermal dose study in rabbits (confidential, 1985 / A6_3_2(1))

Parameter	Control		4 mg/kg		20 mg/kg		100 mg/kg		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	7	7	7	7	7	7	7	7		
Mortality	2	0	2	0	3	2	3	2	–	–
Clinical chemistry										
alkaline phosphatase (ALP)	–	–	–	–	–	–	–	↓*	–	+
At application site										
Encrustration	0	0	0	0	4	4	7	7	+	+
Exfoliation	0	0	0	0	1	0	4	2	+	+
Firm	0	0	0	0	1	1	2	1	+	+
Dark	0	0	0	0	1	0	0	1	–	–
Thickened	0	0	0	0	1	1	4	2	+	+
Ulceration	0	0	0	0	0	0	2	1	+	–

In a **3-week dermal study in rabbit** (confidential, 1985 / A6_3_2(2), key study, doses: 0, 10, 40, 160 mg/kg bw/day) a dose-dependent increase in histopathological changes in the kidneys of female rabbits treated with 40 and 160 mg/kg bw/day of chlorophene was observed (Table18) The changes included tubular calcinosis, tubular proliferation and cellular infiltration. There were five rabbits in each group with one showing histopathological changes in the unexposed group whereas four and five showed histopathological changes in the groups given 40 and 160 mg/kg bw/day, respectively. Moreover, the severity of the histopathological findings increased with dose. Measurements of kidney-associated clinical chemistry and urinalysis parameters were not statistically different between control and treated animals although histopathological changes were observed. It is reported that serum creatinine and urea levels were not changed until there is a 50% reduction in renal function (Price, 2002). Liver weights were reduced in females at the highest dose (160 mg/kg bw/day) and reduced levels of plasma ALP were found in both sexes. Local reactions to treatment were observed in all animals treated with ≥ 40 mg/kg bw/day.

Table 18: Nephropathy in rabbits (confidential, 1985 / A6_3_2(2),)

Animal **M=male** **F=Female**	**0** **mg/kg bw/day**	**10** **mg/kg bw/day**	**40** **mg/kg bw/day**	**160** **mg/kg bw/day**
M1	Iz1 Tp2	NA	0	Tc3
M2	Iz3 Tp2	NA	Tc2	0
M3	Iz1	NA	Iz1	0
M4	0	NA	Iz1	Iz3
M5	0	NA	0	Tu:Di2
F1	0	NA	Tc2	Tc3
F2	0	NA	0	Tp3 Ix1 Pl1 Va1
F3	0	NA	Iz3 Tp2	Tu:Pl1
F4	0	NA	Tc2	Tp1 Lz3
F5	Iz1 Tp2	NA	Tc2 Tp2	Tc1

Iz=Infiltration zellulär (cellular infiltration); Tp=Tubuläre proliferation (Tubular proliferation); Tc= Tubuläre calcinose (Tubular calcinosis); Pl=protein in lumen; Va=Vacuolen im zytoplasma (cytoplasmic vacuolisation): Tu:Pl=Tubuli:Preotein in lumen; The numbers indicate grade of severity; NA=not assessed; 0=None detected

The results from these subacute studies indicate that **dermal exposure to chlorophene induces kidney lesions involve histopathological changes in the kidneys of female rabbits leading to a suggested LO(A)EL at 40 mg/kg bw/day** (3-week study, confidential, 1985 / A6_3_2(2)). **The subacute dermal NO(A)EL for systemic effects in rabbits is 25 mg/kg bw/day based on no systemic effects observed** in the 4-week dermal study (confidential , 1989). The skin lesions at the application site observed in all the subacute dermal studies are readily explained by the irritant properties of the test material. The NO(A)EL for local skin effects in rabbits is 1 mg/kg bw/day based on the occasional skin effects observed at 5 mg/kg bw/day in the 4-week study (confidential, 1989).

In Beagle dogs, a subchronic oral study was conducted (administration in gelatine capsules; confidential 1973b / A6_4_1(2), key study, doses: 0, 10, 30, 100, (200) mg/kg bw/day). This study followed a study design that is similar to OECD 409, but with several deviations. No GLP statement was presented as the study was performed prior to the enactment of the GLP regulations. No specification of the test material Santophen I (chlorophene) was provided and information about the purity and stability of the test material was not presented. In this study weight loss was seen in the highest dose group of 200 mg/kg bw/day. This dose group was therefore discontinued after two weeks. Relative liver weights were significantly increased in males at ≥ 10 mg/kg bw/day and in females at ≥ 30 mg/kg bw/day. This increase in liver weight was considered most likely of low toxicological significance. **Relative weights of kidneys were significantly increased in a dose-dependent manner in male dogs at ≥ 30 mg/kg bw/day**. In female dogs relative kidney weights were significantly increased at 100 mg/kg bw/day. Increased kidney-to-brain ratios and

hyposthenuria (i.e. inability to concentrate urine; after 90 days) were seen at 100 mg/kg bw/day in both sexes. The observed kidney effects are likely indicators of early nephropathy and **the subchronic oral NO(A)EL in dogs is therefore 10 mg/kg bw/day**.

In rodents, subchronic and chronic gavage studies were performed under the auspices of the US National Toxicology Program (NTP Technical Report 424, 1994; Birnbaum et al., 1986). Rats were more sensitive to chlorophene than mice. The kidney was the main target organ in rats. In the 95-day study, increased absolute and relative kidney weights and reduced absolute and relative thymus weights were observed at ≥ 240 mg/kg bw/day in females and at 480 mg/kg bw/day in male and female rats. There seemed to be a dose-related increase in incidence and severity of nephropathy in male rats at ≥ 30 mg/kg bw/day, although the severity of the nephropathy was minimal to mild at these doses. Nephropathy of increasing severity (mild to moderate severity) was observed in 240 mg/kg bw/day male rats and in both male and female rats at 480 mg/kg bw/day. The incidence of nephropathy was significantly increased in male rats at ≥ 120 mg/kg bw/day (Birnbaum et al., 1986). These findings were suggested by the applicant not to be of significance since Fischer 344 male rats are known to spontaneously develop nephropathy. However, the concurrent vehicle controls of this study did not develop nephropathy during the course of the study and historical reference data were not included. A subchronic LO(A)EL of 120 mg/kg bw/day and NO(A)EL of 60 mg/kg bw/day for rats in this study is concluded. In the 95-day study mice first responded to treatment at 500 mg/kg bw/day with a decreased kidney weight in males and an increase in liver weight in females. At the same dose reduced body weight gain was observed in male mice. Among both rats and mice, males were more prone to chlorophene-induced nephropathy than females. In addition to the above mentioned repeated-dose toxicity studies a two-generation reproductive toxicity study (OECD 416) was recently conducted in Wistar rats (see section 3.8; confidential, 2008 / A6_8_2(3); doses: 60, 180, 540 mg/kg bw/day by oral gavage). This study was conducted according to internationally accepted guidelines and principles for GLP. In this study male Wistar rats of the parent generation (P generation) were exposed to chlorophene for at least 15 weeks corresponding to ~105 days. The LO(A)EL in this study was at the lowest dose tested, 60 mg/kg bw/day, based on increased absolute and relative kidney weights and microscopic kidney lesions (nephropathy, dilated tubules, basophilic tubules and lymphocytic infiltration) in P-males. **A two-year gavage study in mice** (NTP Technical Report 424, 1994, key study for carcinogenesis, 0; 120; 240; 480 mg/kg bw/day), with 3- and 15-month interim sacrifices revealed time- and dose-related increased severity and incidence of nephropathy in male and female mice, at occurring as early as 3 months after the beginning of chemical administration. Absolute kidney weights of high-dose males were lower than those of the controls at the 3-month interim evaluation. At the 15-month interim evaluation the absolute and relative kidney weights of dosed male mice were lower than those of the controls, as were the absolute kidney weights of dosed females. Kidney weights of all dosed males and females of the mid- and high dose group remained significantly reduced at the 2-year sacrifice. The final mean body weights of all dosed males and mid- and high dose females were lower than those of the controls. A chronic LO(A)EL of 120 mg/kg bw/day for mice were thus concluded based on severity and incidence of nephropathy and changes in kidney weights, whereas a conclusion on a NO(A)EL could not be taken due to effects on the lowest dose tested. **A two-year gavage study in rats** (NTP Technical Report 424, 1994, key study, 0; 30; 60; 120 mg/kg bw/d), with 3- and 15-month interim sacrifices revealed severe, time- and dose-related nephropathy in male and female rats, occurring as early as 3 months after the beginning of chemical administration. In male rats dosed for as long as 2 years, secondary hyper–parathyroidism developed, with

parathyroid gland hyperplasia, mineralization of the kidney and glandular stomach, and fibrous osteodystrophy occurring in the high-dose group. At the 3-month interim evaluation performed during this chronic gavage study absolute and relative kidney weights of male rats receiving 120 mg/kg bw/day and female rats receiving 240 mg/kg bw/day of chlorophene were significantly higher than those of the vehicle controls. Kidney damage is a common finding in aging male rats. However, since the kidney effects observed after chlorophene treatment are present in both male (Fischer 344 and Wistar) and female rats as well as in other species (rabbit, dog and mice), the observed kidney effects are not considered male rat specific. A NOAEL of 60 mg/kg bw/day in male Fischer rats in the 95-day study was concluded (kidney effects). However, in male Wistar rats in the 2-generation study the kidney effects was observed at the lowest dose tested (60 mg/kg bw/day), hence a **subchronic oral LO(A)EL of 60 mg/kg bw/day was concluded upon. The chronic oral NO(A)EL was not possible to determine since the LO(A)EL for rodents is proposed to be at the lowest dose tested, 30 mg/kg bw/day**, based on kidney effects at 30 mg/kg bw/day observed in male rats at 15 month interim evaluation and at the end of the two-year gavage study.

4.6.1 Summary and discussion of repeated dose toxicity

In rats, the target organ for chlorophene is the kidney. Increased kidney weights were noted in the **16-day oral gavage study** in male rats at ≥ 125 mg/kg bw/day. Nephropathy was observed in all animals of the highest dose group. The incidence and severity of nephropathy increased with dose. In mice, liver weights, but not kidney weights, were significantly increased at ≥ 250 mg/kg bw/day in an analogous subacute oral gavage study.

Three **subacute dermal studies** were conducted in rabbits. In the second 3-week study (confidential, 1985 / A6_3_2 (2)) a dose-dependent increase in histopathological changes was observed in the kidneys of female rabbits at ≥ 40 mg/kg bw/day of chlorophene. Moreover local reactions to treatment were observed in all animals treated with ≥ 40 mg/kg bw/day. In the first 3-week study (confidential, 1985 / A6_3_2(1)) local skin effects were observed in animals treated with ≥ 20 mg/kg bw/day of chlorophene. A tendency towards increased incidence and severity of kidney lesions was observed in female rabbits at the time of death (dead/killed during study + sacrificed at termination). In an additional 4-week study (confidential, 1989) slight to moderate skin effects were observed at 5 mg/kg bw/day and moderate to severe skin effects were observed at 25 mg/kg bw/day. Based on the results of these studies, **the subacute dermal NO(A)EL for systemic effects in rabbits is 25 mg/kg bw/day based on the kidney effects observed in female rabbit at 40 mg/kg bw/day (LO(A)EL)**. The NO(A)EL for local skin effects in rabbits is 1 mg/kg bw/day based on the slight to moderate skin effects observed at 5 mg/kg bw/day.

A series of **subchronic** and chronic **oral gavage studies in rodents** were performed. Rats were more sensitive to chlorophene than mice. The kidney was the main target organ in rats. Mice first responded to treatment with an increase in liver weight in female mice and a decreased kidney weight in male mice. Among rats and mice, males were more prone to chlorophene-induced nephropathy compared to the females. In Fischer 344 male rats there seemed to be a dose-related increase in incidence and severity of nephropathy at ≥ 30 mg/kg and the incidence of nephropathy was significantly increased at ≥ 120 mg/kg bw/day in the 95-day study, whereas increased absolute and relative kidney weights and microscopic kidney lesions were observed at 60 mg/kg bw/day with exposure duration of equivalent length in male Wistar rats in the 2-generation study. In addition to

the subchronic studies described, a **two-year oral study** was performed in rats (NTP). At the 3-month interim evaluation performed, absolute and relative kidney weights of male rats receiving 120 mg/kg bw/day and female rats receiving 240 mg/kg bw/day of chlorophene were significantly higher than those of the controls. In addition, severe, time- and dose-related nephropathy was observed in male and female rats. Based on the kidney effects observed in the male Wistar rats in the 2-generation study a **subchronic oral LO(A)EL for rodents of 60 mg/kg bw/days was concluded.** Moreover, **a chronic oral LO(A)EL for rodents of 30 mg/kg bw/day was concluded**, based on kidney effects at 30 mg/kg bw/day observed at the end of the two-year gavage study.

In a **subchronic oral study in Beagle dogs** (administration in gelatine capsules), weight loss was seen in the highest dose group (200 mg/kg bw/day). Relative weights of kidneys were significantly increased in a dose-dependent manner in male dogs at ≥ 30 mg/kg bw/day. In female dogs kidney weights were significantly increased at 100 mg/kg bw/day. Hyposthenuria was observed after 90 days in both sexes at 100 mg/kg bw/day. Based on the observed kidney effects, the **subchronic oral NO(A)EL for dogs was 10 mg/kg bw/day.**

4.6.2 Comparison with the CLP criteria

After evaluation of the studies on repeated-dose toxicity, a classification for specific target organ toxicity - repeated exposure (STOT-RE) is suggested for chlorophene based on the kidney effects observed in rats, dogs and rabbits**.**

According to CLP based on

- Fischer 344 rats in the oral 2-year study with a LO(A)EL of 30 mg/kg bw/day, and
- the 2-generation study in Wistar rats equivalent to a 90-day repeated-dose study with a LO(A)EL at the lowest dose tested of 60 mg/kg bw/day, and
- the 90-day study in Beagle dogs with a LO(A)EL of 30 mg/kg bw/day,

a classification as STOT-RE 2 is warranted. However, due to the results from a 3-week dermal study in rabbits STOT-RE 1 is proposed:

- The 3-week dermal study in rabbits with a LO(A)EL at 40 mg/kg bw/day warrants classification in category 1 (STOT-RE 1), after adjusting the LO(A)EL due to the study length according to CLP Annex 1 3.9.2.9.5.

  The classification as STOT-RE 1 is in correspondence with CLP Annex 1, 2.9.2.7.3. stating:

  Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, haematology, clinical chemistry, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be lifethreatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

  Via undernote (d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

4.6.3 Conclusions on classification and labelling:

The cut-off values for STOT RE Cat 1 are ≤ 60 mg/kg bw/d in the CLP regulation (in a 28-day dermal repeated dose study). Regarding the dose levels leading to toxicity as well as the quality of the findings it can be concluded that chlorophene is subject to classification for specific target organ toxicity - repeated exposure (STOT-RE) Category 1, based on the kidney effects observed in rabbits. **Regulation (EC) No 1272/2008 (CLP): STOT-RE 1; H372 Causes damage to kidneys through prolonged or repeated exposure**

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

In rats, the target organ for chlorophene was the kidney. Increased kidney weights were noted in a 16-day oral gavage study (1982) in male rats at ≥ 125 mg/kg bw/day. Nephropathy was observed in all animals of the highest dose group and the incidence and severity of this increased with dose. In mice, liver weights, but not kidney weights, were significantly increased at ≥ 250 mg/kg bw/day in an analogous sub-acute oral gavage study (1982).

Three sub-acute dermal studies were conducted in rabbits [1989, 1985 A6_3_2(1) and 1985 A6_3_2 (2)]. In the second 3-week study [1985 A6_3_2 (2)], a dose-dependent increase in histopathological changes was observed in the kidneys of female rabbits at ≥ 40 mg/kg bw/day of chlorophene. Moreover, local reactions to treatment were observed in all animals treated with ≥ 40 mg/kg bw/day. In the first 3-week study [1985 A6_3_2(1)], local skin effects were observed in animals treated with ≥ 20 mg/kg bw/day of chlorophene. A tendency towards increased incidence and severity of kidney lesions was observed in female rabbits at the time of death (dead/killed during the study and those sacrificed at termination). In an additional 4-week study (1989), slight to moderate skin effects were observed at 5 mg/kg bw/day and moderate to severe skin effects were observed at 25 mg/kg bw/day.

A series of sub-chronic and chronic oral gavage studies in rodents were performed. Rats were more sensitive to chlorophene than mice. While the kidney was the main target organ in rats, mice first responded to treatment with an increase in liver weight in female mice and a decreased kidney weight in male mice. Among rats and mice, males were more prone to chlorophene-induced nephropathy compared to females. In Fischer 344 male rats there seemed to be a dose-related increase in incidence and severity of nephropathy at ≥ 30 mg/kg bw/day and the incidence of nephropathy was significantly increased at ≥ 120 mg/kg bw/day in the 95-day study, whereas increased absolute and relative kidney weights and microscopic kidney lesions were observed at 60 mg/kg bw/day with an equivalent exposure duration in male Wistar rats in the 2-generation study. In addition to the sub-chronic studies described above, a two-year oral study was performed in rats (1994). At the 3-month interim evaluation, absolute and relative kidney weights of male rats receiving 120 mg/kg bw/day and female rats receiving 240 mg/kg bw/day of chlorophene were significantly higher than those of the controls. In addition, severe time- and dose-related nephropathy was observed in male and female rats.

In a sub-chronic oral study in Beagle dogs (from 1973), weight loss was seen in the highest dose group (200 mg/kg bw/day). Relative weights of kidneys were significantly increased in a dose-dependent manner in male dogs at ≥ 30 mg/kg bw/day. In female dogs kidney weights were significantly increased at 100 mg/kg bw/day. Hyposthenuria (inability to concentrate urine) was observed after 90 days in both sexes at 100 mg/kg

bw/day.

After evaluation of the studies on repeated-dose toxicity, a classification for specific target organ toxicity - repeated exposure (STOT RE) was suggested for chlorophene based on the kidney effects observed in rats, dogs and rabbits**.**

According to the DS, classification with STOT RE 2 was warranted based on the following studies:

- 2-year oral study in Fischer 344 rats with a LO(A)EL of 30 mg/kg bw/day
- 2-generation study in Wistar rats (equivalent to a 90-day repeated-dose study) with a LO(A)EL at the lowest dose tested of 60 mg/kg bw/day
- 90-day study in Beagle dogs with a LO(A)EL of 30 mg/kg bw/day

However, based on the following study, classification with STOT RE 1 is proposed by the DS:

- 3-week dermal study in rabbits with a LO(A)EL at 40 mg/kg bw/day

The classification as STOT RE 1 is in line with CLP Annex 1, 2.9.2.7.3.

Therefore, the DS proposed classification with STOT RE 1 (H372: Causes damage to kidneys through prolonged or repeated exposure).

Comments received during public consultation

One MSCA was in agreement with the classification for STOT RE 1. Two MSCAs and one manufacturer agreed with classification for specific target organ toxicity but considered that category 2 was more appropriate. Two MSCAs questioned whether the effects observed in the 21-day dermal study were severe enough to justify classification as STOT RE 1. One of these MSCAs and the manufacturer commented that classification should be carried out using a weight-of-evidence of approach and that classification should not just be solely based on one result from a short-term dermal study in rabbits.

The manufacturer provided a more detailed analysis of the study with effects warranting classification as STOT RE 1. Local irritating effects were observed at 40 mg/kg bw/day starting on the first day of application. At day 5, all animals of this dose group exhibited skin reddening and oedema prior to and after treatment. At termination, all animals of this group showed skin lesions. The animals of the 160 mg/kg bw/day treatment group showed such strong lesions that the application area was changed several times during the treatment period. It was therefore considered that the animals of these treatment groups suffered from the repeated application due to the irritating properties of the substance. With regards to the treatment effects, the manufacturer highlighted that nephrotoxic effects such as cellular infiltration (grade 1 -3) and tubular proliferation (grade 2) were recorded in all animals, including 4/9 control animals, with 1 male dying on day 5. In the 40 mg/kg bw/day group, nephrotoxicity occurred in 7/10 animals, with cellular infiltration and tubular proliferation of the same grade as in the control animals. The only additional finding in the kidney was tubular calcinosis (grade 2) in 3 of the affected kidneys. Urinalysis, serum creatinine and urea levels remained unchanged in all treated rabbits, suggesting that no functional changes had occurred.

One MSCA questioned whether chlorophene should be classified at all for STOT RE due to a lack of quantitative data and histopathological data, particularly relating to the hyposthenuria reported in dogs. The DS provided additional information during the

public consultation (see the BD).

Additional key elements

The DS provided the following information, regarding the 90-day study in Beagle dogs. Weight loss was observed in the highest dose group of 200 mg/kg bw/day and at 100 mg/kg bw/day there was significantly lower body weight gain in both females and males. Relative kidney weights were significantly increased in a dose-dependent manner in male dogs and at 100 mg/kg bw/day in females. No clinical observations were reported to support that the dogs were dehydrated. Urine volume was not reported. Clinical chemistry values were normal and water consumption was not reported. The results from urine analysis were also normal. The observed low specific gravity of the urine may originate from polydipsia caused by symptoms from other organs. No observations were reported to support this possibility.

Assessment and comparison with the classification criteria

Chlorophene has been tested for repeated dose toxicity by the oral route in mice, rats and dogs and in dermal studies in rabbits. The most significant effects observed throughout these studies were to the kidneys. The key findings are summarised in the table below:

Table. Severe and significant effects observed in animals at doses relevant for classification as STOT RE 1 and STOT RE 2.

Study Design	**Severe Effects**		**Other Significant Effects**		**No Adverse Effects**
	At doses relevant for classification as STOT-RE 1	At doses relevant for classification as STOT-RE 2	At doses relevant for classification as STOT-RE 1	At doses relevant for classification as STOT-RE 2	
ORAL EXPOSURE					
Mouse ($B6C3F_1$), 16-day, gavage	N/A*	None	N/A	Increased liver weight† in females ≥ 250 mg/kg bw/day and at 500 mg/kg bw/day in males Nephropathy‡ in 40 % of mice at 500 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 62.5, 125, 250, 500 mg/kg bw/day)**	62.5, 125 mg/kg bw/day
Mouse ($B6C3F_1$), 95-day, gavage	N/A	None	N/A	None **(doses relevant for classification with STOT-RE 2: 30, 60 mg/kg bw/day)**	30, 60 mg/kg bw/day
Mouse, ($B6C3F_1$), 95-day, gavage	N/A	N/A	N/A	N/A	Effects were seen at all doses (all of which were above those relevant for classification

Mouse, ($B6C3F_1$), 2-year, gavage	N/A	N/A	N/A	N/A	Effects were seen at all doses (all of which were above those relevant for classification
Rat (F344), 16-day, gavage	N/A	None	N/A	Increased kidney weight in males at doses ≥ 125 mg/kg bw/day and females at ≥ 500 mg/kg bw/day Dose-dependant mild – moderate nephropathy at doses ≥ 62.5 mg/kg bw/day (70 % incidence at ≥ 500 mg/kg) Increased liver weight in males ≥ 250 mg/kg bw/day Decreased thymus weight in females at doses ≥ 250 mg/kg bw/day and in males at ≥ 500 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 62.5, 125, 250, 500 mg/kg bw/day)**	Effects were seen at all doses
Rat, (Wistar), two-generation reproduction study, gavage	N/A	None	N/A	Dose-dependent increase in nephropathy in P an F1 males at ≥ 60 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 60 mg/kg bw/day)**	Effects were seen at all doses
Rat (F344), 95-day, gavage	N/A	None	N/A	Dose-dependant nephropathy at ≥ 30 mg/kg bw/day (minimal to mild severity) **(doses relevant for classification with STOT-RE 2: 30, 60 mg/kg bw/day)**	Effects were seen at all doses
Rat (unstated), 112-day, diet	N/A	None	N/A	None **(doses relevant for classification with STOT-RE 2: 10.6, 39.4 mg/kg bw/day)**	10.6, 39.4, 115.4 mg/kg bw/day

Rat (F344), 2-year, gavage	N/A	N/A	N/A	N/A	Effects were seen at all doses (all of which were above those relevant for classification
Dog (Beagle), 21-day, capsule	None	None	None **(doses relevant for classification with STOT-RE 1: 3, 10, 30 mg/kg bw/day)**	Lower overall body weight gain at 100 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 100 mg/kg bw/day)**	3, 10, 30 mg/kg bw/day
Dog (Beagle), 90-day, capsule	N/A	None	N/A	Reduced body weight gain at 100 mg/kg bw/day Absolute kidney weight increased in males at 100 mg/kg bw/day Relative kidney weight increased in males at ≥ 30 mg/kg bw/day in males and at 100 mg/kg in females Relative liver weight increased in males at ≥ 10 mg/kg bw/day and females at 30 mg/kg bw/day Specific gravity significantly reduced at 100 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 10, 30, 100 mg/kg bw/day)**	Effects were seen at all doses
DERMAL EXPOSURE					
Rabbit (NZW), 5-day, dermal	None	None	None **(doses relevant for classification with STOT-RE 1: 2.4, 60, 125 mg/kg bw/day)**	None **(doses relevant for classification with STOT-RE 2: 500 mg/kg bw/day)**	2.4, 60, 125 and 500 mg/kg bw/day

Rabbit (NZW), 21-day, dermal	None	None	None **(doses relevant for classification with STOT-RE 1: 4, 20 mg/kg bw/day)**	Kidney lesions at 100 mg/kg bw/day Liver lesions more common amongst decedents Decreased alkaline phosphatase in females at 100 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 100 mg/kg bw/day)**	4, 20 mg/kg bw/day
Rabbit (HC:NZW), 21-day, dermal	None	None	Tubular calcinosis of the kidney at 40 mg/kg bw/day (females only) **(doses relevant for classification with STOT-RE 1: 10, 40 mg/kg bw/day)**	Increased incidence of histopathological changes in the kidney at 160 mg/kg bw/day (see table 5) Decreased liver weights in females at 160 mg/kg bw/day Local reactions to treatment in all animals at ≥ 40 mg/kg bw/day **(doses relevant for classification with STOT-RE 2: 160 mg/kg bw/day)**	10 mg/kg bw/day
Rabbit (NZW), 28-day, dermal	None	N/A	Local skin effects at 25 mg/kg bw/day (erythema, atonia, discolouration) **(doses relevant for classification with STOT-RE 1: 5, 25 mg/kg bw/day)**	N/A	1 and 5 mg/kg bw/day
INHALATION EXPOSURE					
There were no repeated dose studies carried out by the inhalation route					

* N/A – Not applicable, there were no doses in range for this subcategory of STOT-RE

† Details on the magnitude of weight gain and reduction of animals and organs were generally unavailable

‡ Details on severity of effects observed were generally unavailable

Summary of renal findings in each species:

Oral Administration:

Mice

Chlorophene was administered to mice by gavage for 16 days, 95 days and 2 years. Nephropathy, categorised by multifocal tubule dilation, flattening of the proximal convoluted tubule epithelium, tubule regeneration and minimal focal epithelial cell necrosis was observed at doses relevant for classification with STOT RE 2 only in mice of the 16-day study. However, in longer term studies, these effects were not observed at

doses relevant for classification.

Rats
Chlorophene was administered to rats by gavage for 16 days, in a 2-generation reproduction study (equivalent to 90 days), for 95 days and for 2 years. It was also administered to rats via the diet for 112 days. The main renal effects observed at doses relevant for classification occurred in the 16 day, 90 day and 95 day studies were nephropathy, which was generally mild to moderate in severity and dose-dependent, and increased kidney weight in both males and females. These effects only occurred at doses relevant for classification with STOT RE 2. Whilst nephropathy did occur in the chronic study, all doses used were above those relevant for classification with STOT RE 2. No renal effects were observed in the dietary study at any doses.

Dogs
Two capsule studies were available in dogs, one of 21-days duration and one of 90 days. No renal effects were observed during the 21-day study. In the 90-day study, increased relative kidney weight was observed in male dogs at all doses and in females at the top dose only. Hyposthenuria (the inability to concentrate urine) was observed in both sexes at the top dose. These effects were suggested to be indicative of early nephropathy and occurred only at doses relevant for classification with STOT RE 2.

Dermal Administration:
Rabbits
Chlorophene was administered to the skin of rabbits in 4 studies: 5-days, 21 days (2 studies) and 28 days. Kidney lesions were generally observed at doses ≥ 100 mg/kg bw/day. There was also evidence of an increased incidence and severity of histopathological lesions at 160 mg/kg bw/day (Table). These doses are relevant for classification with STOT RE 2. The only finding in the kidney relevant for classification with STOT-RE 1 was an increased incidence of grade 2 tubular calcinosis in females at 40 mg/kg bw/day (3/5 versus 0/5 in controls) [observed in the second of two 3-week rabbit studies 1985 A6_3_2(2)]. This effect was not observed in the first 3-week dermal study in rabbits [1985 A6_3_3(1)] dosed at up to 100 mg/kg bw/day. Urinalysis parameters, serum creatinine and urea levels remained unchanged, indicating a lack of functional change in the kidneys.

Table. Breakdown of effects observed in the kidneys of Rabbits following dermal administration of chlorophene for 3 weeks.

	Males				**Females**			
	0	10*	40*	160**	0	10*	40*	160**
Cellular Infiltration	3/5	NA	2/5	1/5	1/5	NA	1/5	2/5
Tubular Proliferation	2/5	NA	0/5	0/5	1/5	NA	2/5	2/5
Tubular Calcinosis	0/5	NA	1/5	1/5	0/5	NA	3/5	2/5
Protein in Lumen	0/5	NA	0/5	0/5	0/5	NA	0/5	1/5
Cytoplasmic Vacuolisation	0/5	NA	0/5	0/5	0/5	NA	0/5	1/5
Tubuli: Protein in Lumen	0/5	NA	0/5	0/5	0/5	NA	0/5	1/5

NA – Not assessed, animals of these groups were not assessed for histopathology
*Doses relevant for classification with STOT-RE 1

**Doses relevant for classification with STOT-RE 2

Inhalation Administration:
There were no studies carried out by the inhalation route and as such, no assessment can be made on specific target organ toxicity by this route.

Conclusion:
Chlorophene has been tested in mice, rats and dogs via repeated oral administration. It has also been tested in 4 studies in rabbits via the dermal route.

According to CLP, classification with category 1 for STOT RE is on the basis of significant and/or severe toxic effects, of relevance to human health, produced at generally low exposure concentrations. In the studies provided, the only effect occurring at a dose relevant for classification with STOT RE 1 was an increased incidence of tubular calcinosis in female rabbits, in a 21-day dermal study (3/5 rabbits versus 0 in controls). The severity was graded as weak to medium (grade 2) and the finding was not replicated in any of the other studies. In addition, there were no changes in clinical chemistry or urinalysis values between the groups raising into question the significance of this effect.

Therefore, RAC concluded that classification of chlorophene as STOT RE 1 is not justified and instead a weight-of-evidence approach as required under CLP should be used.

On the basis of increased incidence of nephropathy and increased kidney weight in rodents after oral administration, and in rabbits after dermal administration of chlorophene, **RAC concludes that chlorophene should be classified as STOT RE 2 (H372:May cause damage to kidneys through prolonged exposure).**

4.7 Germ cell mutagenicity (Mutagenicity)

In-vitro genotoxicity of chlorophene

Table 19: *In-vitro* genotoxicity of chlorophene

Test system, Method, Guideline	Organism/ strain(s)	Concentrations tested	Results		Remarks	Reference
			– S9	+ S9		
Ames test: Salmonella/ Microsome test No guideline, but ≅ OECD 471 Non-GLP	*S. typhimurum* TA98, TA100, TA1535, TA1537	0.1, 0.3, 1.0, 3.0, 10.0, 33.0, 66.0, 100.0 µg/plate (-S9 and +S9)	Neg. based on no increased mutation frequencies in the tested strains. The study did not include the recommended test strain (TA102) to reveal mutagenicity at A:T basepairs or of agents with crosslinking potential. The four strains used for testing reveal mutagenesis at G.C basepairs only.	Neg. based on no increased mutation frequencies in the tested strains. The study did not include the recommended test strain (TA102) to reveal mutagenicity at A:T basepairs or of agents with crosslinking potential. The four strains used for testing reveal mutagenesis at G.C basepairs only.	Cytotoxicity at ≥ 33 µg/plate (– S9) and 100 µg/plate (+S9)	Mortelmans *et al.*, 1986 A6_6_1 (1) **KEY STUDY**
Cytogenicity (Chromosomal	CHO cells	–S9: 4, 8, 15, 30 and 60	Neg. based on no increase in	Neg. based on no increase in	Complete inhibition of	Confidential , 1994

Test system, Method, Guideline	Organism/ strain(s)	Concentrations tested	Results		Remarks	Reference
			– S9	+ S9		
aberrations) in Chinese hamster ovary (CHO) cells No guideline, but ≅ OECD 473		µg/mL +S9: 1.3, 2.5, 5, 10 and 20 µg/mL	chromosome aberrations. Several study shortcomings: Only 100 of the required 200 metaphases were scored, insufficient incubation times with test compound with S9, no study with continuous treatment to confirm a negative result	chromosome aberrations. Several study shortcomings: Only 100 of the required 200 metaphases were scored, insufficient incubation times with test compound with S9, no study with continuous treatment to confirm a negative result	mitotic index at ≥ 30 µg/mL without S9, and at 20 µg/mL with S9	A6_6_2 **KEY STUDY**
Sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells No guideline Non-GLP	CHO cells	–S9: 0.5, 1.6, 5.0, 16.0 µg/mL +S9: 0.05, 0.16, 0.5, 1.6, 5.0, 16.0 µg/mL	Neg. based on that the SCE frequency did not increase with >= 20% above the concurrent solvent control level which was chosen as a statistically conservative positive response. Fifty second-division metaphase cells were scored at each dose level.	Neg. based on that the SCE frequency did not increase with >= 20% above the concurrent solvent control level which was chosen as a statistically conservative positive response. Fifty second-division metaphase cells were scored at each dose level.	Cytotoxicity: the highest non-lethal dose tested was 16.0 µg/mL	National Toxicology Program (1994) A6_7 (2b)
Mutagenicity test (HPRT) in murine lymphoma cells OECD 476 Exposure time: 3h Cytotoxicity determination: percent relative survival (% RS) adjusted for loss of cells during the 3h exposure period. Mutant frequency (Mf) is expressed as	Mouse L5178Y cells	Exp. 1: 0, 2.5, 5, 10, 20, 25, 30, 35 µg/mL Exp. 2: 0, 5, 10, 20, 22.5, 25, 27.5, 30 µg/mL	Equivocal based on positive findings in on experiment (Exp. 1) that were not confirmed inn a second experiment (Exp. 2). A concentration-related increased mutant frequency (Mf) was observed in Exp. 1 at 2.5-10 µg/mL, compared to control values. The Mf at 10 µg/mL was	Neg. based on increased Mfs at concentrations eliciting unacceptably high levels of cytotoxicity. A concentration related increased Mf up to 20 µg/mL with significantly increased Mf at 20 µg/mL were observed in Exp 2. The relative survival was however <50 % at	Cytotoxicity: >= 50% cytotoxicity at ≥ 25 µg/mL (–S9), ≥ 20 µg/mL (+S9)	Confidential, 2005 A6_6_3 (1) **KEY STUDY**

Test system, Method, Guideline	Organism/ strain(s)	Concentrations tested	Results		Remarks	Reference
			– S9	+ S9		
number of mutant cells per number of surviving cells			statistically different from the control, at recommended cytotoxicity levels of 10-20%.The tests for linear trend were not significant. The finding was not confirmed in Exp. 2. Exp. 1 -S9 Treatment -Mf – %RS 0-4,84-100 2,5-6,3-96,98 5-7,22-95,64 10-11,41-88.47* 20-6,95-75,01 25-5,19-26,92 30-7,84-3,99 35-(10,16)-0,00 Exp. 2 -S9 Treatment -Mf – %RS 0-2,20-100 5-3,30-100,27 10-2,73-81,42 20-2,68-64,19 22,5-4,54-57,01 25-1,08-36,35 27,5-2,74-14,09 30-6,52-1,53	20 µg/mL. A test for linear trend was significant in Exp.2. This positive finding was not confirmed in Exp. 1. Exp. 1 +S9 Treatment -Mf – %RS 0-10,82-100 2,5-10,21-95,77 5-9,53-100,47 10-6,52-57,94 20-11,91-44,00 25-15,07-45,54 30-3,47-17,36 35-9,85-14,83 Exp. 2 -S9 Treatment -Mf – %RS 0-6,05-100 5-2,18-100,08 10-9,76-93,53 20-10,93-40,17* 25-6,29-47,31 27,5-9,99-31,47 30-8,48-8,42 35-5,88-1,48 + Linear trend test significant		
Mutagenicity test ($TK^{+/-}$) in mouse L5178Y and human TK6 cells No guideline Non-GLP L5178Y: Soft agar Incubation time -S9 : 4 h TK6: Microwell plates Incubation time -S9: 20 h Only fast growing mutants (large colonies),	Mouse L5178Y cells Human TK6 cells	L5178Y: 0, 10, 20, 25, 35, 45 µg/mL TK6 cells: 0, 10, 20, 30, 40 µg/mL	Equivocal in mouse L5178Y cells, An concentration-related increased Mf above control level in mouse L5178Y cells starting at the lowest concentration (10 µg/mL) tested, a conc. with satisfactory relative total growth (RTG; >=50%). L5178Y cells Treatment -Mf – RTG 0-48-100 10-55-ND	/	Reduction in relative total growth (RTG) ≥ 50% at 20 µg/mL for L5178Y, and at 40 µg/mL for TK6.	Caspary *et al.*, 1988 A6_6_3 (2) **KEY STUDY**

Test system, Method, Guideline	Organism/ strain(s)	Concentrations tested	Results		Remarks	Reference
			– S9	+ S9		
, i.e. not chromosomal mutants, were recorded. Cytotoxicity : Relative cell growth/plating efficiency during expression			20-90-50 25-110-40 35-130-30 45-240-10 According to recommended global evaluation factors the increased Mf at 10 and 20 µg/mL relative to the vehicle/control levels are too low to allow the recommended trend analyses. Uninformative in human TK6 cells. Background Mf too low compared to the recommended assay acceptance criteria. Increased mutant frequencies in human TK6 cells at cons. >= 40 µg/mL with significantly reduced RTG. TK6 cells Treatment -Mf – RTG 0-25-100 10-60-99 20-50-97 30-45-95 40-80-20			

In vivo

Table 20: In vivo genotoxicity of chlorophene

Type of test, Method/ Guideline	Species, Strain, Sex, no/group	Frequency / route of application	Sampling times	Dose levels	Results	Reference
Micronucleus assay OECD 474	Mice CD-1 ♂ + ♀ 5-15/sex Vehicle control:	Single dose, oral	24, 48 and 72 h	500, 1000, 2000 mg/kg (♂) 250, 500, 1000 mg/kg (♀)	Neg. based on that the frequency of micronucleated polychromatic (immature) and mature erythrocytes were generally similar to those in concurrent vehicle controls at	Confidential, 1990 A6_6_4 (1) **KEY STUDY**

Type of test, Method/ Guideline	Species, Strain, Sex, no/group	Frequency / route of application	Sam-pling times	Dose levels	Results	Reference
	15♂, 15♀ 250 mg/kg: 5 ♀ 500 mg/kg: 5 ♂, 5 ♀ 1000 mg/kg: 5 ♂, 15 ♀ 2000 mg/kg: 15 ♂ Positive control: 5 ♂, 5 ♀				any sampling time. There were no indications of bone marrow toxicity. The individual proportions of immature/mature cells were unaffected by treatment. The statistical test used was the Mann-Whitney U-test. Males (2) and females (1) in the highest dose groups showed signs of toxicity with hunched posture, piloerection, closed eyes and reduced motor activity and were sacrificed *in extremis*. The vehicle control Mfs (mean micronucleated cells/1000) for immature erythrocytes were 0,7 and 0.4 for males and females, respectively and the levels of treated animals never exceeded 1.8 (males and females) compared with the level induced by the positive control (chlorambucil) of 57.8 (males) and 50.7 (females).	
Dominant-lethal test No guideline Non-GLP	Mice ♂ no further details	Single dose, i.p.	-	100, 200 mg/kg	Neg. based on that chlorophene did not induce dominant lethal mutations in germ cells of male mice.	Confidential, 1972 A6_6_4 (2)
In vivo comet assay No guideline "Quasi" guideline in Tice et al., 2000 Single Cell Gel/Comet Assay: Guidelines for In Vitro and In Vivo Genetic Toxicology Testing. *Envir. And Mol. Mutagenesis*, **35**, p206-221. GLP	Mice ICR (CD-1) ♂ Preliminary toxicity test: 2/dose Main test: 7/dose	Two doses: the first at 0h and the second at 20h. Gavage	4h and 24 h after initial dose	Initial preliminary toxicity test using DMSO as solvent: 200, 300, 400, 2000 Preliminary toxicity test using corn oil as solvent: 200, 300, 400, 2000 mg/kg bw Main test using corn oil as solvent: 90, 180, 360 mg/kg bw	Neg. based on no increased DNA damage levels in the organ tested. Mortality was observed with DMSO as solvent at 400 mg/kg. The solvent was changed to corn oil and mortality was observed at 2000 mg/kg. The experiment did not include studies of the target organ (site of contact) for chlorophene, the kidney, as specified to the industry prior to onset of the experiment. There was no evidence of an increase in the percentage tail intensity values in single cell/nuclei suspensions from liver and glandular stomach, or bone marrow from animals dosed with the test item dose	Confidential, 2009 A6_6_5 **KEY STUDY**

Type of test, Method/ Guideline	Species, Strain, Sex, no/group	Frequency / route of application	Sam-pling times	Dose levels	Results	Reference
					groups when compared to the concurrent vehicle control group. The positive control produced an acceptable increase in the percentage tail intensity value in all tissues scored. The presence of clinical signs indicated that systemic absorption had occurred.	

4.8.3 Summary and discussion of mutagenicity

Conclusion on the genotoxicity of chlorophene was equivocal, in the *in vitro* tests in bacteria and mammalian cells.

Several of the *in vitro* studies (Mortelmans et al., 1986;confidential, 1994 / A6_6_2) exhibit study insufficiencies that reduce their power to conclude with chlorophene being not genotoxic. In two independent *in vitro* mutagenicity studies in mammalian cells (mouse L5178Y cells) assessing mutagenesis in two different loci (HPRT and TK) there were indications of increased mutation frequencies without metabolic activation (confidential, 2005 / A6_6_3(1); Caspary et al., 1988). The first study is a well-conducted study following OECD Guideline 476 (1997), and the latter study is a non-guideline, non-GLP $TK^{+/-}$ assay conducted with chlorophene of unknown specification (Caspary et al., 1988).

In vivo there were no indications of clastogenicity or aneugenicity in the micronucleus assay in mice. The systemic availability of the test compound to the bone marrow is however in general questioned with negative results in micronucleus test, particularly when no bone marrow toxicity was observed in any of the exposed groups. An agreement with the applicant for conducting a second *in vivo* genotoxicity assay (*in vivo* comet assay) in mice was made (confidential, 2009 / A6_6_5). However assessment of genotoxic effect in the target organ, the kidney, was not performed, leaving the study of low power to conclude on the potential genotoxic properties of chlorophene in relevant tissues. The comet assay was negative for the tested organs. The dominant-lethal test (confidential, 1972 / A6_6_4 (2)) is only available as a summary in which a negative result is reported.

No systematic search for structure-activity relationships to known germ cell mutagens were conducted.

4.8.4 Conclusions on classification and labelling

In summary, several of the key studies exhibit study insufficiencies that hamper establishment of solid conclusions on genotoxicity, but based on an overall evaluation of the available data using a *Weight of Evidence* approach the decision on the genotoxicity is negative.

No classification is proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Chlorophene was tested in a number of *in vitro* and *in vivo* mutagenicity studies. Several of the *in vitro* studies exhibited methodological deficiencies that reduced their reliability to conclude on the genotoxicity of chlorophene. In two independent studies using mammalian cells (mouse L5178Y cells) assessing mutagenesis at two different loci (HPRT and TK), there were indications of increased mutation frequencies, in the absence of metabolic activation. *In vivo*, there were no indications of clastogenicity or aneugenicity in a micronucleus assay in mice. Systemic availability was questioned due to a lack of bone marrow toxicity in any of the exposed groups. An *in vivo* comet assay was carried out in mice, which was negative for the tested organs, however the target organ, the kidney was not tested, making it difficult to conclude on the potential genotoxic properties of chlorophene in relevant tissues.

The DS concluded that although several of the key studies had deficiencies which made it difficult to make a solid conclusion, the overall weight of evidence suggested that chlorophene was negative for genotoxicity.

Comments received during public consultation

One MSCA commented specifically that they agreed with no classification for mutagenicity, although this position was not further elaborated.

Assessment and comparison with the classification criteria

The potential mutagenicity of chlorophene has been studied *in vitro* in both bacteria and mammalian cells, and *in vivo* in a mouse micronucleus test, a comet assay and a mouse dominant lethal test.

In an Ames test from 1986, clear negative results were seen in *S.typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without S9. A test for chromosome aberrations in Chinese hamster ovary cells also gave a negative result with and without S9. Sufficiently high top dose levels were used to achieve a complete inhibition of mitotic index. Neither of these tests conformed to the relevant guidelines available today, but still they do not provide any indications that chlorophene has mutagenic potential.

The results from 2 mammalian cell tests were less clear cut. In a study dated 1988, as part of a wider testing programme for the US National Toxicology Programme, a positive-dose-related trend in mutant fraction (MF) was seen at the TK locus in mouse lymphoma cells following 4 h exposure in soft agar without S9. However, apparently a detailed trend analysis was not possible given the extent of the data, and the test was neither repeated nor conducted with S9. This study also included a single mutation test using human TK cells in microwell plates. However the results were uninterpretable given that the background MF was below the recommended assay acceptance criteria. In the other study, a HPRT mutation test dated 2005, the methodology apparently conformed to OECD TG 476. Without S9, in a first experiment, the MF at each dose tested was above the control value, but a clear dose-response was not seen and a significant increase was only evident at one of the mid-dose values. This finding was not reproduced in a second experiment. With S9, there was no increase in MF in the first experiment but when repeated elevated values were seen at several doses and a positive linear trend test reported. However, overall, these assays do not appear to have provided a clear indication of mutagenic potential.

The results of the three *in vivo* tests provide further reassurance that chlorophene lacks mutagenic potential. The micronucleus test (1990) was well performed, giving a clear negative result in male and female mice treated orally. The Dominant Lethal test (1972) was non-guideline, but gave a negative result. The comet assay (2009) investigated DNA isolated from bone marrow, liver and glandular stomach of male mice treated orally. This was a well conducted test and also gave a clear negative result.

RAC concludes that in the absence of any positive results, and given the range of tests conducted, **no** germ cell mutagenicity classification for chlorophene is justified.

4.8 Carcinogenicity

Table 21: Carcinogenicity studies

Type of study, Method/ Guideline	**Species, Strain, Sex, no/group**	**Dose levels, frequency of application**	**Tumours**	**Reference**
Two-year gavage study US-EPA 83-2 ≅ OECD 453	Rat F344/N ♂+♀ 50/sex 3-month and 15-month interim sacrifice: 10/sex	0, 30, 60, 120 mg/kg/day (♂) 0, 60, 120, 240 mg/kg/day (♀)	♂**: No evidence** of carcinogenic effect in ♂ receiving 30, 60, or 120 mg/kg day. ♀**: Equivocal evidence** of carcinogenic effect in ♀ based on the occurrence of <u>two very rare renal transitional cell carcinomas; one occurring at 120 mg/kg/day and one at 240 mg/kg /day.</u> No changes in survival or mean body weights in control or exposed groups.	National Toxicology Program Technical Report Series No.424 (1994) A6_5+A6_7 **KEY STUDY***
Two-year gavage study US-EPA 83-2 ≅ OECD 451	Mouse $B6C3F_1$ ♂ + ♀ 50/sex 3-month and 15-month interim sacrifice: 10/sex	0, 120, 240, 480 mg/kg/day	♂**: Some evidence** of carcinogenic effect in ♂ based on increased incidences of renal tubule adenoma in high dose and renal tubule adenoma or carcinoma (combined) at mid- and high-dose.♀**: No evidence** of carcinogenic activity in ♀. Survival of high-dose male and female mice was lower than that of the controls. The number of mice surviving to the end of the study was considered adequate for evaluation of chronic toxicity and carcinogenicity. Final mean body weights of all dosed males and mid- and high dose females were lower than those of the controls.	National Toxicology Program Technical Report Series No.424 (1994) A6_7 (1) **KEY STUDY***
One-year dermal initiation/promotion study No guideline	Mouse Swiss CD-1 ♂ + ♀ 50/sex	As initiator: single application of 10 mg/animal As promoter: 0.1, 1.0, 3.0 mg/animal, 3 times/week As complete carcinogen: 10 mg single initiating dose followed by 0.1, 1.0, 3.0 mg/animal 3 times/week	No skin tumour-initiating potential. **Weak tumour-promoting activity**. There was a dose-related increased incidence in papillomas in both males and females. Chlorophene did not act as a complete carcinogen.	National Toxicology Program (1995) A6_7 (3)
20-week dermal carcinogenicity study in transgenic	Mouse Tg.AC mice	0.1, 1.0, 3.0 mg/animal	**A significant ($p<0.01$) carcinogenic effect on skin** were recorded at 3 mg/animal	Spalding JW, French, Tice,

Type of study, Method/ Guideline	Species, Strain, Sex, no/group	Dose levels, frequency of application	Tumours	Reference
Tg.AC mice No guideline	♀ 13-20 animals/group	3 times per week Post-exposure period: 6-10 weeks	measured as incidence of animals with tumours analysed by life-table analysis. **Tumour multiplicity was also significantly (p<0.01) increased** in the high dose group. The mean latency time to maximum skin tumour yield was the same for the negative and positive control groups and for the highest chlorophene dose group. Survival at 20 weeks decreased with increasing doses (87%, 77% and 68% in the low-, medium and high-dose groups, respectively) No overt skin irritation and ulceration was reported after monitoring by gross examination.	Furedi-Machacek, Haseman and Tennant. Tox. Sci., 49, 241-254, 1999 A6_7 (2) **KEY STUDY**

*Studies also reported in Table 3.7 (chronic toxicity).

The evaluation of carcinogenicity is based on four studies, see Table above.

The carcinogenic potential of chlorophene was studied in **two-year gavage studies in rats and mice**. The studies were conducted by the National Toxicology Program.

Rats, gavage, two year (NTP):

In rats, very rare renal transitional cell carcinomas occurred in one female rat each of the mid- and high dose group (table 4.14) whereas none of the tumours found in male rats could be ascribed as an effect of the test substance (table 4.15**)** Due to the low incidence, this finding is considered as **equivocal evidence** for a carcinogenic activity of the test compound in female rats. Survival and mean body weights of dosed animals were similar to those of the controls. Non-neoplastic endpoints comprised severe time- and dose-related nephropathy in male and female rats, occurring as early as 3 months after the beginning of chemical administration (females). In male rats dosed for as long as 2 years, secondary hyper-parathyroidism was observed, with parathyroid gland hyperplasia, mineralization of the kidney and glandular stomach, and fibrous osteodystrophy occurring in the high-dose group. The severity of these lesions was greater in males.

Table 22: Nephropathy and pathological findings in female rats

Dose (mg/kg)	0		60		120		240	
3-Month Interim Evaluation								
Kidney[a]	10		10		8		9	
Nephropathy[b]	1	(0.1)[c]	3	(0.3)	3	(0.4)	7**	(1.2)**
15-Month Interim Evaluation								
Kidney	10		10		10		10	
Nephropathy	9	(0.9)	10	(1.2)	9	(1.1)	10	(1.8)**
2-Year Evaluation								
Kidney	50		50		51		50	
Nephropathy	46	(1.2)	47	(1.2)	50	(1.5)*	50	(2.4)**
Single Sections (Standard Evaluation)								
Kidney	50		50		51		50	
Renal Tubule Hyperplasia	0		0		0		1	
Renal Tubule Adenoma[d]	0		0		0		1	
Transitional Cell Carcinoma[e]	0		0		1		1	
Step Sections (Extended Evaluations)								
Kidney	50		–[f]		–		50	
Renal Tubule Hyperplasia	2		–		–		2	
Single and Step Sections combined								
Kidney	50		–		–		50	
Renal Tubule Hyperplasia	2		–		–		3	
Renal Tubule Adenoma	0		–		–		1	
Transitional Cell Carcinoma	0		–		–		1	

* Significantly different (P≤0.05) from control group by Fisher exact test; severity significantly different by Mann-Whitney U test
** P≤0.01
[a] Number of animals with kidney examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesion in all animals: 0=none, 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year corn oil gavage studies with vehicle control groups: 2/1,068 (1.9% ± 0.2%); range 0%-2%
[e] Historical incidence: 0/1,068
[f] Animals not examined in extended or combined evaluations

Table 23: Nephropathy and pathological findings in male rats

Dose (mg/kg)	0		30		60		120	
3-Month Interim Evaluation								
Kidney[a]	10		10		10		9	
Nephropathy[b]	10	(1.0)[c]	8	(0.8)	9	(1.0)	8	(0.9)
15-Month Interim Evaluation								
Kidney	10		10		10		9	
Nephropathy	10	(1.7)	10	(2.1)°	10	(2.1)°	9	(2.2)°
2-Year Evaluation								
Kidney	50		49		50		50	
Nephropathy	48	(2.3)	48	(2.8)°	48	(2.9)°°	50	(3.3)°°
Single Sections (Standard Evaluation)								
Kidney	50		49		50		50	
Renal Tubule Hyperplasia	0		2		0		2	
Renal Tubule Adenoma	1		0		0		0	
Renal Tubule Carcinoma	0		0		0		1	
Renal Tubule Adenoma or Carcinoma[d]	1		0		0		1	
Step Sections (Extended Evaluations)								
Kidney	50		49		50		50	
Renal Tubule Hyperplasia	3		7		6		17°°	
Renal Tubule Adenoma	0		1		2		1	
Renal Tubule Carcinoma	0		0		0		1	
Renal Tubule Adenoma or Carcinoma	0		1		2		2	
Single and Step Sections combined								
Kidney	50		49		50		50	
Renal Tubule Hyperplasia	3		9		6		17°°	
Renal Tubule Adenoma	1		1		2		1	
Renal Tubule Carcinoma	0		0		0		1	
Renal Tubule Adenoma or Carcinoma	1		1		2		2	

° Significantly different (P≤0.05) from control group by Fisher exact test; severity significantly different by Mann-Whitney U test
°° P≤0.01
[a] Number of animals with kidney examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesion in all animals: 0=none, 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year corn oil gavage studies with vehicle control groups: 12/1,069 (1.1% ± 1.4%); range 0%-4%

Mice, gavage, two year (NTP):

In mice following exposure to chlorophene by gavage, there was some evidence of carcinogenic effect in males based on significant increase in renal tubule adenomas in the 480 mg/kg (high-dose) group of males and of renal tubule adenomas or carcinoma in both the 240 (mid-dose) and 480 mg/kg (high-dose) groups of males (table 25).

Table 24: Nephropathy and pathological findings in male mice

Dose (mg/kg)	0	120	240	480
3-Month Interim Evaluation				
Kidney[a]	10	10	10	10
Nephropathy[b]	1 (0.1)[c]	3 (0.3)	10°° (1.2)°°	10°° (2.2)°°
15-Month Interim Evaluation				
Kidney	10	10	10	10
Nephropathy	9 (0.9)	10 (2.7)°°	10 (2.7)°°	10 (2.7)°°
2-Year Evaluation				
Kidney	50	50	50	50
Nephropathy	39 (0.8)	48°° (2.0)°°	50°° (2.4)°°	49°° (2.4)°°
Single Sections (Standard Evaluation)				
Kidney	50	50	50	50
Renal Tubule Hyperplasia	0	0	3	6°°
Renal Tubule Adenoma[d]	0	2	2	2
Renal Tubule Carcinoma[e]	0	0	2	1
Renal Tubule Adenoma or Carcinoma[f]	0	2	4°	3
Step Sections (Extended Evaluations)				
Kidney	50	50	50	50
Renal Tubule Hyperplasia	9	16°	13	9
Renal Tubule Adenoma	0	1	2	3
Renal Tubule Carcinoma	0	0	1	0
Renal Tubule Adenoma or Carcinoma	0	1	3	3
Single and Step Sections Combined				
Kidney	50	50	50	50
Renal Tubule Hyperplasia	9	16°	14	13
Renal Tubule Adenoma	0	2	4	5°
Renal Tubule Carcinoma	0	0	2	1
Renal Tubule Adenoma or Carcinoma	0	2	6°°	6°°

° Significantly different (P≤0.05) from control group by Fisher exact test; severity significantly different by Mann-Whitney U test
°° P≤0.01
[a] Number of animals with kidney examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesion in all animals: 0=none, 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year corn oil gavage studies with vehicle control groups (mean ± standard deviation): 4/949 (0.4% ± 1.0%); range 0%-2%
[e] Historical incidence: 0/949
[f] Historical incidence: 4/949 (0.4% ± 1.0%); range 0%-2%

Tubular hyperplasia was seen in all treated groups with significant increase in low-dose males but without a dose-response. This study featured gavage administration five days per week of 120, 240, 480 mg/kg bw/day of chlorophene in corn oil to B6C3F1 mice. Treatment was continued for 2 years, with interim sacrifices at 13 and 65 weeks. The 2-year sacrifice comprised 50 animals per sex and dose, whereas each interim sacrifice comprised 10 animals per sex and dose. General toxicity was evident as nephropathy (table 4.16) and reduced body weight development (table 4.17). The

incidence and severity of nephropathy were both age- and dose-related. Nephropathy was first statistically significant in the mid-dose group at the 13-week sacrifice with 10 out of 10 males (8/10 females; 240 mg/kg) all showing minimal nephropathy. At the 65-week sacrifice, nephropathy had progressed to a mild-to-moderate degree and was seen in all treated groups with a dose-related increase in severity of nephropathy and affecting both males and females. Nephropathy was evident as interstitial fibrosis, multifocal dilated tubules with flattening of the renal tubule epithelium, regenerative tubules with basophilic epithelium, thickened basement membranes and hyaline casts.

Table 25: Body weights and absolute and relative organ weights of mice

	Vehicle Control	120 mg/kg	240 mg/kg	480 mg/kg
Male				
n	45	32	38	30
Necropsy body weight	48.0 ± 1.0	39.1 ± 0.9°°	35.5 ± 0.7°°	32.6 ± 0.7°°
Brain				
Absolute	0.453 ± 0.004	0.465 ± 0.005	0.473 ± 0.008°	0.469 ± 0.005°[b]
Relative	9.66 ± 0.24	12.08 ± 0.27°°	13.48 ± 0.32°°	14.51 ± 0.29°°[b]
L. Kidney				
Absolute	0.399 ± 0.008	0.263 ± 0.005°°	0.253 ± 0.006°°	0.267 ± 0.007°°
Relative	8.45 ± 0.22	6.80 ± 0.15°°	7.20 ± 0.21°°	8.26 ± 0.25
R. Kidney				
Absolute	0.415 ± 0.006	0.280 ± 0.005°°	0.266 ± 0.006°°	0.281 ± 0.005°°
Relative	8.80 ± 0.20	7.24 ± 0.15°°	7.57 ± 0.21°°	8.71 ± 0.23
Liver				
Absolute	2.424 ± 0.117	2.398 ± 0.202	1.949 ± 0.078°	2.113 ± 0.056°
Relative	51.25 ± 2.73	64.56 ± 7.14	55.66 ± 2.80	65.35 ± 1.95°
Female				
n	36	40	33	25
Necropsy body weight	47.2 ± 1.3	44.9 ± 1.2	40.4 ± 1.2°°	33.7 ± 0.9°°
Brain				
Absolute	0.477 ± 0.004	0.473 ± 0.003	0.465 ± 0.003°	0.462 ± 0.005°
Relative	10.39 ± 0.32	10.85 ± 0.31	11.88 ± 0.39°°	13.94 ± 0.44°°
L. Kidney				
Absolute	0.277 ± 0.004	0.261 ± 0.005	0.231 ± 0.004°°	0.206 ± 0.014°°
Relative	5.99 ± 0.17	5.96 ± 0.20	5.90 ± 0.21	6.35 ± 0.72
R. Kidney				
Absolute	0.291 ± 0.005	0.276 ± 0.005°	0.250 ± 0.005°°	0.214 ± 0.006°°
Relative	6.29 ± 0.16	6.29 ± 0.20	6.38 ± 0.22	6.43 ± 0.22
Liver				
Absolute	1.970 ± 0.077	2.223 ± 0.123	2.208 ± 0.090	2.121 ± 0.089
Relative	42.38 ± 1.66	52.14 ± 4.24°	56.67 ± 3.53°°	63.13 ± 2.34°°

° Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
°° P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=29

The survival of chlorophene-treated animals decreased following chlorophene exposure (table 4.18). Survival of dosed male mice was lower than that of controls (90%) with 69% (p=0.014), 81% (p=0.222) and 64% (p=0.002) in the low-, mid- and high dose groups, respectively. Survival of female mice was 74% for controls and 85%, 69% (p=0.665) and 51% (p=0.007) in the low-, mid- and high-dose groups, respectively. The number of mice surviving to the end of the study was

considered adequate for evaluation of chronic toxicity and carcinogenicity. The decreased survival was associated in part with dose-related increases in incidence and severity of nephropathy. The mean body weights at necropsy of all dosed males and mid- and high-dose females were lower than those of the controls. Liver and kidney weights were dose-dependently increased in both sexes.

Table 26: Survival of mice

	Vehicle Control	120 mg/kg	240 mg/kg	480 mg/kg
Male				
Animals initially in study	70	70	70	70
3-Month interim evaluation[a]	10	10	10	10
15-Month interim evaluation[a]	10	10	10	10
Moribund	2	12	7	8
Natural deaths	3	4	5	10
Accidental deaths[a]		2		2
Animals surviving to study termination	45	32	38	30
Percent probability of survival at end of study[b]	90	69	81	64
Mean survival (days)[c]	591	551	572	530
Survival analysis[d]	P=0.007	P=0.014	P=0.222	P=0.002
Female				
Animals initially in study	70	70	70	70
3-Month interim evaluation[a]	10	10	10	9
15-Month interim evaluation[a]	10	10	10	9
Moribund	9	4	10	12
Natural deaths	5	3	5	14
Accidental deaths[a]		3	2	1
Animals surviving to study termination	36	40	33	25
Percent probability of survival at end of study[b]	74	85	69	51
Mean survival (days)[c]	583	554	551	520
Survival analysis[d]	P<0.001	P=0.314N	P=0.665	P=0.007

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A lower mortality in a dose group is indicated by N.

Mice, dermal:

Female transgenic Tg.AC mice carrying an inducible v-Ha-*ras* oncogene were exposed to 0.1, 1.0, 3.0 mg/animal by the dermal route 3 times per week for 20 weeks followed by a 6 week post-exposure period (Spalding et al., 1999; A6_7 (2)), (table 4.19) They used 13-20 animals/group at the age of 7-18 weeks. Doses of 14.3, 143, 429 µg/cm² BPA solved in acetone (0.5, 5, 15 mg/mL concentrations, total volume of 0.2 mL/animal) were applied to a dorsal interscapular skin area of 5-7 cm². Positive control used was 12-O-tetradecanoylphorbol-13-acetate (TPA). Body weights were recorded weekly and cage observations were made twice a day for mortality and morbidity. Detailed clinical observations 2-5 times weekly. Overt skin irritation and ulceration was monitored by gross examination. Numbers of skin tumours were recorded weekly. Evaluations of gross

appearance of epidermal papillomas were done, and in order to be counted as a positive response, skin tumours had to reach 1 mm in size and persist for at least 3 weeks. Once an equivocally positive skin response to either the test agent or positive control (TPA) had been established, further observations became unnecessary. Survival decreased in a dose-dependent manner at 20 weeks with 87%, 77% and 68% in the low-, medium and high-dose groups, respectively. **A significant (p<0.01) carcinogenic effect was recorded** at 3 mg/animal measured as the incidence of animals with tumours, analysed by life-table analysis. **Tumour multiplicity was also significantly (p<0.01) increased** in the high-dose group. The mean latency time to maximum tumour yield was the same for the negative and positive control groups and for the high-dose group.

Table 27: Skin Tumour Incidence in homozygous female Tg.AC mice

Treatment	Incidence of animals with tumors (%)	Mean weeks to first tumor (± SD)	Mean tumors/animals at risk (± SD)[a]	Mean weeks to maximal tumor yield (± SD)	Survival at 20 weeks (%)
Acetone	4/14 (29%)	13.3 ± 2.1	0.3 ± 0.0	13.3 ± 2.1	14/14 (100%)
BCP[b]					
0.1 mg	5/15 (33%)	8.6 ± 6.1	0.7 ± 1.2	16.0 ± 1.9	13/15 (87%)
1.0 mg	1/13 (8%)	16.0	0.1	16.0	10/13 (77%)
3.0 mg	16/19 (84%)[c]	10.9 ± 3.6	2.3 ± 1.9[d]	13.5 ± 3.6	13/19 (68%)
TPA[e]					
1.25 µg	19/20 (95%)[c]	7.4 ± 2.4	19.5 ± 12.4[d]	13.6 ± 3.5	9/20 (45%)

Note. Mice were 18 weeks old at start of treatment.
[a] Animals were considered to be at risk after 10 weeks of dosing.
[b] o-Benzyl-p-chlorophenol was dissolved in acetone and applied 3 times per week
[c] $p < 0.01$ vs. acetone controls (Life Table Test).
[d] $p < 0.01$ vs. acetone controls (Mann-Whitney U-Test).
[e] TPA (1.25 µg) in 200 µL acetone was applied twice per week.

In a study with male and female CD-1 mice the potential to **initiate or promote skin tumours** was investigated in a 20 week dermal initiation/promotion study using phorbol ester (TPA) or dimethyl-benz-anthracene (DMBA) as model promoter and initiator, respectively (NTP, 1995, A6_7 (3)). **Chlorophene acted as a weak skin tumour promoter**, and did not demonstrate activity as a skin tumour initiator or a complete carcinogen.

4.8.1 Summary and discussions of carcinogenicity

Rats, gavage, two year (NTP):

In the 2-year study (table 4.14 and table 4.15), F344 rats received chlorophene by gavage administrations of 30, 60, 120 (males) or 60, 120, 240 (females) mg/kg bw/day in corn oil. Treatment was continued for 2 years, with interim sacrifices at 13 and 65 weeks. The 2-year sacrifice comprised 50 animals per sex and dose, whereas each interim sacrifice comprised 10 animals per sex and dose. General toxicity was restricted to kidney effects (nephropathy and effects on urine parameters).

The findings in female rats included very rare carcinomas of the renal transitional epithelium (TCC). One female each of the mid- and high-dose group were found to carry this type of tumour. This type of tumour has not been recorded in NTP historical control data (0/1068). The rarity of this tumour type raises concern, since the tumour occured twice in this study, which reduces the possibility that the tumours occurred by chance. The likelihood that the two TCCs observed in this study are of spontaneous origin is very low. Considerations of tumour type and incidence of tumour

type relative to historical control incidence is highlighted in the R7 Guidance to CLP (3.6.2.2.6):"*Historical control data can also be useful to judge the biological significance of marginal increases in uncommon tumours. If there is a small increase in a particular tumour type which historical data shows to be very uncommon and unlikely to have occurred by chance then this may support a conclusion of carcinogenicity without the requirement for a statistically significant increase.*"

Another consideration is the evaluation of whether there is any indication that the observed tumours are substance related, such as increased incidences of pre-neoplastic lesions. A review was conducted within the NTP study to specifically evaluate the transitional cell hyperplasia. This review of high-dose and vehicle control rats from the 15-month interim evaluation and 2-year study was limited to the transitional epithelium lining, the renal pelvis, and papilla. An increased incidence of transitional cell hyperplasia was detected in both high-dose females and males after 15 months and 2 years (the two time points not differentiated in the report). Thus, the observed tumours were accompanied by increases in pre-neoplastic lesions, but the existence of hyperplasias *per se* is not on its own predicitive of the development of tumours. Sex-differences seem to exist.

Moreover, since the tumour type (Transitional cell carcinoma) is relevant for humans, their occurrence should be included in the overall evaluation of the carcinogenicity of chlorophene.

Mice, gavage, two year NTP: It is logical to speculate that the renal hyperplasias and neoplasms observed in male mice following orally administered chlorophene are secondary to the nephrotoxicity exerted by the test compound. As discussed in Marsman et al., 1995, previous studies in mice with other chemicals, both genotoxic and non-genotoxic

(bromochloromethane CAS number 74-27-5; nitrilotriacetic acid CAS number 139-13-9; tri(2,3-dibromopropyl)phosphate CAS number 126-72-7; 2,4-diaminophenol dihydrochloride CAS number 137-09-7) showed poor association between nephropathy and renal carcinogenicity. Thus, although nephropathy may be a permissive factor, other primary and secondary mechanisms may be operative in the induction of the mouse renal neoplasms.

In lifetime bioassays compounds are routinely tested using at least three dose levels to enable hazard identification and hazard characterisation as part of risk assessment. Of these doses, the highest dose is meant to provoke minimal toxicity, characterised by overt toxicity, toxicity believed to reduce the life span or an approximately 10% reduction in body weight gain (maximal tolerated dose, MTD dose). The MTD is the highest dose of the test agent during the bioassay that can be predicted not to alter the animal's normal longevity from effects other than carcinogenicity. In the current study the low-dose males express sufficient toxicity to qualify as an actual high-dose group, and in the low-dose animals there is an increase in renal neoplasms, and in the mid- and high-dose the increase is statistically significant. Thus there appears to be a dose-response relationship between the dose of chlorophene and the development of renal neoplasms, starting at the lowest dose. The increase in renal tubule hyperplasia was also significantly increased in the low-dose males. Altogether these findings support a classification of the test compound in Category 2 (According to **Regulation (EC) No 1272/2008 (CLP)**.

Other compounds have been classified as Category 2 carcinogens on the basis of tumours occurring at doses at and exceeding MTD, in which the incidence of tumours showed a dose-response relationship (*e.g.*, Polyhexamethylene biguanide– PHMB, CAS number 27083-27-8). The data on genotoxicity were limited, and PHMB was concluded as non-genotoxic.

Mice, dermal: Transgenic female Tg.AC mice

The study in the transgenic Tg.AC mice suggests that chlorophene is carcinogenic, although dose-dependent decreased survival was observed at 20 weeks. Dermal dosing of female Tg.AC mice with 3 mg chlorophene in acetone over 20 weeks led to a significant increase of skin tumours (84% of animals had tumours) compared to the acetone control (29%).

Considerations of the relevance of the model used, for studies with regulatory purposes:

As stated in R7.7.10 of the document "Guidance on information requirements and chemical safety assessment R.7a: Endpoint specific guidance" related to the REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006, data from transgenic rodent models can be used for assessing the carcinogenicity of a chemical: ***"Genetically engineered (transgenic) rodent models*** *(e.g., Xpa-/-, p53+/-, rasH2 or Tg.AC): animals can be genetically engineered such that one or more of the molecular changes required for the multi-step process of carcinogenesis has been accomplished (Tennant et al., 1999). This can increase the sensitivity of the animals to carcinogens and/or decrease the latency with which spontaneous or induced tumours are observed. The genetic changes in a given strain of engineered animals can increase sensitivity to carcinogenesis in a broad range of tissues or can be specific to the changes requisite for neoplastic development in one or only a limited number of tissues (Jacobson-Kram, 2004; Pritchard et al., 2003; ILSI/HESI 2001). Data from these models may be used in a Weight of Evidence analysis of a chemical's carcinogenicity."*

Although studies of carcinogenicity using the Tg.AC model are not yet fully validated for regulatory purposes, extensive investigations have already been conducted. In the study with chlorophene included here (Spalding et al., 1999) several compounds were tested and the results showed good correlation with results from 2-year bioassays, with 3/6 compounds including chlorophene showing clear carcinogenic activity, and 3/6 that were negative in Tg.AC mice, of which one non-genotoxic compound have shown to induce liver tumours in female mice in the 2-year bioassay. A more extensive array of compounds have been tested in the Tg.AC model and the results have been compared with results from the 2-year bioassay (Eastin et al., 1998; Pritchard et al., 2003), showing good concordance, and that the model is able to detect also non-genotoxic carcinogens.

4.9.2 Comparison with the CLP criteria

The "Guidance on the Application of Regulation (EC) No 1272/2008" (Guidance to CLP) 3.6.2.2.6 lists important factors, which may be taken into consideration when assessing the overall level of concern of possible carcinogenic compounds. In the following sections chlorophene have been evaluated in relation to these factors:

(a) tumour type and background incidence; The observed tumour types (renal transitional cell carcinomas in female rats, the renal tubular adenomas and carcinomas in male mice and skin tumours in mice) are relevant for humans, and they arose from histologically unrelated tissues, although vicinal, suggesting that chlorophene may lead to more than one type of tumour.

With respect to the two TCCs observed in female rats, the Guidance to CLP states "If there is a small increase in a particular tumour type which historical data shows to be very uncommon and unlikely to have occurred by chance then this may support a conclusion of carcinogenicity without the requirement for a statistically significant increase".

(b) multi-site responses; It is stated in the Guidance to CLP: "*Evidence shows that substances which cause tumours in either multiple sites and/or multiple species tend to be more potent carcinogens than those causing tumours at only one site in one species (Dybing et al., 1997). This is often true for substances which are mutagenic. Also, where human carcinogens have been tested in two or more species, the majority have caused cancer in several species (Tennant, 1993). Thus, if a substance causes tumours at multiple sites and/or in more than one species then this usually provides strong evidence of carcinogenicity.*"

The kidney tumours appeared at two different, but vicinal sites, and they arose from histologically unrelated tissues and occurred in two species: TCCs in female rats and renal tubular adenomas and carcinomas in male mice. Besides these skin tumours occurred in mice. The occurrence of tumours at different sites suggests that chlorophene may be carcinogenic.

(c) progression of lesions to malignancy; In male mice there seems to be a low but treatment-related increase in benign renal adenomas, but not in malignant renal carcinomas. The number of tumours may be too low for progression from adenomas to carcinomas to be evident.

(d) reduced tumour latency; The latency of tumour development, i.e. how quickly a substance induces tumours, often reflects the potency of a carcinogen. No tumours were found at the interim sacrifices in the studies in mice and rats and the mean latency time to maximum tumour yield was not decreased in Tg.AC mice exposed to chlorophene. In summary, there is no indication for reduced tumour latency due to chlorophene.

As mentioned in the Guidance to CLP, the latency of tumour formation does not materially affect the classification and hazard category. Any substance causing cancer will attract classification regardless of the latency for tumour development. This also includes tumour responses at late treatment/life periods if substance-related.

(e) whether responses are in single or both sexes; Tumours appeared in both sexes, male and female mice and in female rats. The renal tubular adenomas and carcinomas are restricted to male mice, the skin tumours appeared in female mice, whereas the TCCs were only seen in female rats. There are no data on possible differences in toxicokinetics between the sexes. Female mice did not develop adenomas despite almost a similar degree of nephropathy at the highest dose.

(f) whether responses are in a single species or several species; The renal tubular adenomas and carcinomas were observed in mice (oral, males), and the skin tumours were observed in mice (dermal, females), whereas the TCCs were observed in rats (oral, females). Thus, these findings are to be discussed as a several-species response.

It is mentioned in the Guidance to CLP that "a single study in one species and sex in combination with positive in-vivo mutagenicity data would be considered to provide sufficient evidence of carcinogenicity. Positive responses in several species add to the weight of evidence, that a chemical is a carcinogen."

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; No comment added.

(h) routes of exposure; The oral exposure route chosen in the NTP studies is *per se* not relevant to the foreseeable human exposure pattern towards chlorophene. The dermal studies in CD-1 and transgenic mice cover the most relevant human exposure patterns, both test systems showing that chlorophene as a weak carcinogen.

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; This comparison cannot be made due to a lack of relevant human data.

(j) the possibility of a confounding effect of excessive toxicity at test doses; See discussion above, under Mice, gavage, two-years NTP.

Despite evidence of nephropathy in mice and rats of both sexes, no increased incidences of renal neoplasms were observed in female mice or in male rats. This suggests that while nephrotoxicity may have been a necessary component, factors other than the marked nephrotoxicity of chlorophene were critical to the development of renal carcinogenesis in male mice.

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. The genotoxicity of chlorophene was concluded as negative, although some uncertainties with respect to assay insufficiencies existed. The mode of action underlying the development of renal tubular adenomas and carcinomas may involve regenerative hyperplasia. On the other hand as previously discussed studies in mice with both genotoxic and non-genotoxic chemicals, (bromochloromethane CAS number 74-27-5; nitrilotriacetic acid CAS number 139-13-9; tri(2,3-dibromopropyl)phosphate CAS number 126-72-7; 2,4-diaminophenol dihydrochloride CAS number 137-09-7) showed poor association between nephropathy and renal carcinogenicity. Thus, although nephropathy may be a permissive factor, other primary and secondary mechanisms are likely to be operative in the induction of the mouse renal neoplasms.

The aetiology of the TCCs is not clear. The low incidence may not be reflected in the occurrence of precursor lesions in the urothelium, even if there was transitional epithelium hyperplasia in the urinary bladder of one control animal and one high dose animal.

4.9.3 Conclusions on classification and labelling

In view of the induced renal neoplasms in male mice (2-year study) along with the two cases of renal transitional cell carcinoma, not observed in historical animals, in female rats (2-year study), and supported by the carcinogenic effect of chlorophene following dermal exposure of female CD-1 mice or female Tg:AC mice, chlorophene is considered a weak carcinogen. The genotoxic property of chlorophene was concluded as negative and no clear mode of action was established.

A classification for carcinogenicity is proposed:

According to Regulation (EC) No 1272/2008 (CLP): Carcinogen category 2, H351 suspected of causing cancer.

This is based on induction of two different types of tumours, in two species, in two sexes and following two routes:

Rat female (oral) + mice males (oral) + mice (dermal)

Mode of action: No clear mode of action was established

Threshold or non-threshold: The totality of the data available indicates no genotoxicity and threshold.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Two carcinogenicity studies following US EPA guideline 83-2 were available, one in rats and one in mice. Also available as supportive information was a non-guideline dermal initiation/promotion study and a short-term dermal carcinogenicity study in transgenic mice.

In the two-year carcinogenicity gavage study in F344 rats, males were treated orally with 0, 30, 60 or 120 mg/kg bw/day and females with 0, 60, 120 or 240 mg/kg bw/day chlorophene. No effects on survival or mean body weights were seen. In a standard evaluation, one female from the mid dose group and one female from the highest dose group were found to have a rare carcinogenic tumour of the renal transitional epithelium. Historical control data from the US NTP database showed that there were 0 incidences of this tumour out of 1068 controls. This led the DS to conclude that the likelihood that these tumours were spontaneous was low and that the study provided equivocal evidence of carcinogenicity.

In the two-year carcinogenicity gavage study in B6C3F1 mice, animals were treated orally with 0, 120, 240 or 480 mg/kg bw/day chlorophene. At the end of the 2-year experimental period, an extended evaluation was performed using step sections of the kidney. Renal tubule adenomas were observed in male mice, dose-dependently across all study groups, reaching statistical significance at 480 mg/kg bw/day [5/50 (10 %) versus 0 in controls]. Renal tubule carcinoma was evident in two males at 240 mg/kg bw/day [2/50 (4%)] and in one male at 480 mg/kg bw/day [1/50 (2%)]. The incidence of adenoma and carcinoma combined reached statistical significance at doses ≥ 240 mg/kg bw/day. Renal tubular hyperplasia was also observed in all treated groups but in the absence of a dose-response relationship. These effects were observed at doses all greater than the maximum tolerated dose (MTD) with reductions in body weight of 20, 26 and 32 % at necropsy for dose groups 120, 240 and 480 mg/kg bw/day, respectively. However, the DS argued that this level of toxicity did not detract from the conclusions on carcinogenicity arising from the findings, citing other substances considered by RAC in the past (e.g. PHMB: polyhexamethylene biguanide). The DS also noted an increased severity of nephropathy at these doses (grading 0.8, 2.0, 2.4 and 2.4 for 0, 120, 240 and 480 mg/kg bw/day, respectively). No neoplasms were observed in female mice.

Supportive information was available from a 20-week dermal initiation/promotion study in CD-1 mice and a dermal study in transgenic mice.

In the initiation/promotion study, chlorophene (10 mg/animal) was applied topically to 50 female and 50 male Swiss CD-1 mice as an initiator. Repeated topical applications of 0.1, 1 or 3 mg/animal were then applied three times a week for a year. When chlorophene treatment was followed by promotion using the phorbol ester tetradecanoyl phorbol acetate (TPA), chlorophene was not found to exert any initiating activity. However, there was a dose-related increased incidence of papilloma in both males and females following chlorophene treatment after initiation with dimethyl-benz-anthracene (DMBA). In conclusion, chlorophene did not act as a skin tumour initiator or as a complete carcinogen but did have activity as a weak skin tumour promoter.

In the second dermal study, female Tg.AC transgenic mice (13 – 20/group) were dosed dermally with chlorophene (0.1, 1, 3 mg/animal) 3 times per week for over 20 weeks. The results showed a significant increase in skin tumours in animals treated with chlorophene (3 mg/animal) over the vehicle controls (84% versus 29% respectively). Survival decreased at 20 weeks in a dose-dependent manner with 86%, 77% and 68% survival noted in the low, medium and high dose groups, respectively.

The DS concluded that the rare transitional cell carcinoma observed in two rats and the renal neoplasms occurring in male mice supported by the carcinogenic effects occurring in the dermal studies showed that chlorophene was a weak carcinogen. Given that chlorophene was not genotoxic and no clear modes of action had been established for the carcinogenic effects seen, the DS favoured classification as Carc. 2 rather than Carc. 1B.

Comments received during public consultation

Three MSCA and a manufacturer of chlorophene commented during public consultation.

All three MSCA agreed with the proposal. One MSCA queried the relevance of referring to another dossier in relation to the discussion of the presence of neoplasms at doses exceeding the MTD. Another offered further information on historical control incidence of the tumour types observed: transitional cell carcinoma in rats [1/1348 in female rats (0.07%) (Haseman *et al*., 1998) and 0.09% in F344 rats (Chandra *et al*., 1993)] and renal tubule carcinomas in male B6C3F1 mice [1/1351 (0.07%) (Haseman *et al*., 1998) versus 0/949 in the CLH report]. The third MSCA highlighted the significance of the rare tumour findings in female rats. They were less certain about the relevance of the findings in the 2-year mouse study as they occurred at doses causing excessive toxicity.

Further details about the study in transgenic mice were requested, specifically on the dose-dependent reduction in survival (87, 77 and 68% survival in dose groups 0.1, 1.0 and 3.0 mg/animal, respectively). In response, the DS suggested that the reduced survival was due to the advanced age of the mice at the start of the study (18 weeks) and the spontaneous development of odontomas leading to removal of mice from the study.

The manufacturer disagreed with the proposal to classify for carcinogenicity, providing the following comments on the studies in the dossier.

(i) 2-year bioassay in rats:

The evidence that the isolated cases of transitional cell carcinoma (TCC) in one mid-dose female and one top-dose female rat were treatment related was not convincing. As chlorophene was considered non-genotoxic, carcinogenesis would likely occur as a progression from pre-neoplastic lesions to the malignancy. In a review conducted within the carcinogenicity study, renal transitional cell hyperplasia was found to be inversely related to tumour incidence, in that hyperplasia incidence was greater in males. Observations were made in the transitional epithelium lining, the renal pelvis and papilla.

The manufacturer also provided further historical control data for transitional cell tumours in female F344 rats from the NTP database. These were:

- 1 TCC/1348 female F344 rats in chronic feeding studies
- 1 TCC/898 female F344 rats in chronic inhalation studies

These data showed that this tumour type, whilst rare, did occur spontaneously in this strain of rat.

(ii) 2-Year bioassay in mice:

In males, the reported positive effect was based on significantly higher frequency of renal tubule adenoma in the high dose group and of renal tubule adenoma and carcinoma combined in the mid and high dose groups. Importantly, if the carcinoma were analysed alone, they were found neither to follow a dose-dependent pattern nor to be statistically significant (incidence of carcinoma: 0/50, 0/50, 2/50 and 1/50 for control, 120, 240 and 480 mg/kg bw/day groups respectively).

The increased tumours in males occurred as a consequence of chlorophene-induced nephrotoxicity at doses exceeding the MTD. Nephropathy was observed in all dose groups in both male and female mice. No neoplasms occurred in females. However, it was noted that the natural progression of nephropathy proceeds more slowly in female mice and so the incidence and severity was lower than in males.

Industry commented that the low dose was sufficiently toxic to qualify as a high dose group as, in accordance with the relevant test guideline, reductions in body weight of 19% and reduction in kidney weight of 20% were evident. In this group, two adenomas were recorded in males (no statistical significance and within HCD recorded in the NTP database), but no carcinomas were found.

Therefore, industry concluded that chlorophene followed a non-genotoxic mechanism of action by which long term exposure to elevated doses was required for the potential development of tumours. As a significant tumour increase was only observed for benign tumours in one sex at doses exceeding the MTD and carcinoma occurred only in a dose-independent manner without statistical significance, industry agree with classification on the basis of these effects.

(iii) *Dermal initiation/promotion study in mice:*

The weak tumour promotion activity was evident only at the top dose. This activity was much lower than for corresponding DMBA/TPA treated positive control mice and there was no evidence of activity at the two lower doses. Scaling, and/or crusting, ulceration and irritation was also evident in the top dose group. Therefore, it was suggested that the skin irritating properties of chlorophene could cause an increase in keratinocyte turnover. The manufacturer noted that hyperkeratosis was observed in a 3-week pilot study at the same top dose level of chlorophene, with increased incidence and severity after one-year of exposure. It was postulated that such a stimulation of cell proliferation could exert a promoting effect on initiated cells.

The initiation/promotion study was considered by industry to be of limited relevance. Chlorophene was non-promoting at sub-irritant concentrations and there was no evidence of chemical-related increased incidences of neoplasms or non-neoplastic lesions following histopathological examinations of kidney, liver, nose and thymus.

(iv) *20-Week dermal carcinogenicity study in transgenic Tg.AC mice:*

The reliability of this non-guideline study was questioned by industry due to the following reasons:

- The lack of skin irritation could not be explained; this had been seen in other studies using comparable dosing regimen.
- No histopathology was performed in order to determine whether any precursory lesions were present.
- The use of mice that were 18 weeks old rather than 7-8 weeks meant that animals were removed from the study on account of spontaneously occurring jaw tumours. This may have influenced survival numbers.
- The observed papillomas were seen to develop and regress over the course of the study, reducing the significance of the assay as a model for carcinogenesis.
- Disparate substances (including chlorophene) which affected different but only internal tissues in the standard rodent bioassay "*were seemingly*

metamorphosed into skin carcinogens in the Tg.AC model upon their application to the skin" (Ashby 1997).

- The study was given a Klimish score of 3 (not reliable) by the Norwegian Environmental Agency.

Industry concluded that this study did not allow a final conclusion due to its insufficient reliability.

Overall, industry concluded that classification was not justified. They commented that the effects observed were either spontaneous (TCC in rats), or a secondary, non-specific consequence of kidney toxicity (adenoma in mice) or irritancy (papilloma after dermal treatment in transgenic mice).

Additional key elements

Incidences of transitional cell hyperplasia and carcinoma in male and female rats, following administration of chlorophene for 15-months and 2-years are shown in Table below.

Dose (mg/kg bw/day)	**0**	**120**	**240**
Transitional cell hyperplasia (15-month + 2-year combined)			
Males	5/59 (9%)	26/59	-
Females	4/60 (7%)	-	17/59
Transitional cell carcinoma			
Males	0	0	-
Females	0	1/51 (1.9%)	1/50 (2%)

Assessment and comparison with the classification criteria

From four available studies, two carcinogenicity bioassays conducted for the US NTP in rats and mice are considered the most relevant for classification purposes.

In female F344 rats, single incidences of a rare renal tumour type occurred in the mid and top dose groups. No such transitional cell carcinoma was seen in the kidneys of controls or in any male rats. The DS stated that no such tumours had been seen in 1068 control animals, presumably from studies involving treatment by gavage, although this wasn't explicitly stated. In contrast, information provided during the public consultation indicated that transitional cell carcinoma had been seen in control F344 rats from the NTP historical control database, although the incidence rate was very low (1/1348 females in feeding studies; 1/898 females in inhalation studies).

RAC concluded that it was plausible for single incidences of this rare tumour type to occur spontaneously in F344 rats, but noted that in this study the incidence overall was two for this tumour type. There was no mechanistic basis to suggest that the transitional cell carcinomas in female rats in this study were treatment related. There is no evidence of chlorophene being genotoxic and no clear relationship was established between treatment-related toxicity (e.g. renal transitional cell hyperplasia) and susceptibility of animals to this tumour type. RAC considers the evidence for a carcinogenic effect of chlorophene in female rats to be very weak, but it can not be disregarded completely. The conclusion of the DS that the evidence for carcinogenicity in rats was equivocal seems reasonable.

The only potentially significant tumour findings in the B6C3F1 mouse study were seen in males:

- Renal tubule adenoma: 0/50 – 2/50 – 2/50 - 2/50, in control, low, mid, high dose groups.
    - Historical control incidence for 2-year gavage studies was 4/949 and the range 0-2%.
- Renal tubule carcinoma: 0/50 -0/50 – 2/50 – 1/50.
    - Historical control incidence 0/949.

A further microscopic evaluation was made by making "step" instead of "single" sections of the renal tissue:

- Renal tubule adenoma: 0/50 – 1/50 – 2/50 – 3/50
- Renal tubule carcinoma: 0/50 – 0/50 – 1/50 – 0/50
- No historical control data available.

None of these tumour findings are statistically significant. However, when the data from both evaluations are combined and the numbers of adenoma and carcinoma are combined, there is a statistical significance with $P \leq 0.01$ at the mid and top doses; and for adenoma only at the top dose ($P \leq 0.05$).

RAC is unsure of the relevance of the extended evaluations, especially in the absence of any historical control data. When only the data from the standard pathology examination is considered, there is a slight non-statistically significant increase in benign renal tumour incidence. There was no dose-response relationship, although the incidence rate of 2/50 seen in each group was above the historical control rate (0-1/50). Renal tubule hyperplasia was significantly increased in frequency (6/50) and severity at the top dose, but was absent at the low dose and seen in only 3/50 mid dose animals. As such, there was no association between hyperplasia and tumour incidence in the male mice. Nephropathy was common in each group of male mice, but the incidence pattern and severity did equate to the benign tumour findings. The nephropathy was evident as interstitial fibrosis, multifocal dilated tubules with flattening of the renal tubule epithelium, regenerative tubules with basophilic epithelium, thickened basement membranes and hyaline casts. Nephropathy incidence (severity score) was as follows in the control, low, mid and high dose animals: 39/50 (0.8) - 48/50 (2.0) – 50/50 (2.4) – 49/50 (2.4).

According to RAC, it appears plausible that both the slight increase in renal tubule adenoma and the increased renal nephropathy seen in dosed animals may have been treatment related. However, it is unclear whether there was a mechanistic association between the chloroprene-related nephropathy and the increased incidence of adenomas. Similarly, it is unclear whether the relatively high rate of nephropathy seen in control males indicates an inherent, low level increased sensitivity of these mice to renal cancer development during treatment with chlorophene.

The DS considered that the mode of action underlying the development of renal tubular adenomas (and carcinomas) may have involved regenerative hyperplasia. However, they also noted that studies in mice with both genotoxic and non-genotoxic substances have shown poor association between nephropathy and renal carcinogenicity. A clear mechanism has not been established.

Survival of chlorophene-treated mice was lower than controls: end of study survival rates calculated for males and females were 90%, 69%, 81% and 64% and 74%, 85%, 69% and 51%, in controls, low, mid and high dose groups, respectively. The manufacturer argued that the renal tumours seen in males were associated with increased mortality. However, there was no indication that the toxicity that led to increased mortality was the basis for renal tumour development. No such tumours were seen in females at comparable levels of toxicity and mortality.

In conclusion, there was limited evidence of chlorophene carcinogenicity in this study. Increased nephropathy and mortality were related to tumour incidence and no tumours

were observed in females. However, there is no clear mechanistic basis to discount the findings in males; they are of potential relevance to humans.

The two additional cancer studies were of limited relevance and reliability. Both were compromised by limited reporting and a lack of histopathological analysis. Additionally, the assays may have been compromised by the application of doses that were significantly irritant to mouse skin.

According to the CLP criteria, limited evidence of carcinogenicity is sufficient to classify a substance in category 2. In this context, limited evidence can be shown by the tumour findings being seen in only one study, by there being unresolved questions about the interpretation of the results of that study, and by the increased tumours associated with exposure to the test substance being benign or of uncertain neoplastic potential. Additionally, the finding of one TCC in the mid dose group and one TCC in the top dose of females in the rat study cannot be disregarded completely and therefore provides weak supporting evidence for this classification. RAC considers that the mouse bioassay showing an association between renal tumours and exposure to chlorophene also provides limited evidence of carcinogenicity. There is no mechanistic basis to disregard the potential relevance of these tumour findings to humans.

As there are no human carcinogenicity data, classification with category 1A would be inappropriate. Similarly, RAC concluded that a category 1B classification was not supported because there were no consistent tumour findings between rats and mice, or between males and females, the rates of renal tumours in the exposed mice were relatively low and there was a possibility of confounding by excessive toxicity and the only tumour findings in rats were considered of equivocal relevance.

In conclusion, RAC agrees with the DS that the rare transitional cell carcinoma observed in rats and the renal neoplasms occurring in male mice fulfil the criteria for classification as Carc. 2. This is also supported by the lack of a mode of action that would dismiss the relevance to humans.

4.9 Toxicity for reproduction

4.9.1 Effects on sexual function and fertility

Table 28: Effects of chlorophene on sexual function and fertility

Route of exposure	Test type, Method, Guideline	Species, Strain, Sex, no/group	Exposure Period	Doses	Critical effect	NOAEL/ LOAEL Parental	NOAEL/ LOAEL F1	NOAEL F2	Reference
Oral, gavage	One-generation study No guideline, but ≅ OECD 415 Non-GLP	Rat Charles River albino strain 10 ♂+ 20 ♀ per group	♂: 60 days pre-mating, 106 days total ♀: 14 days pre-mating, through-out gestation and lactation	0, 50, 150 mg/kg bw/day	P males in the 150 mg/kg bw/day group gained less body weight during the pre-mating period than the control males. The body weight reduction continued until the end of the study. Females (P) were not affected. Both 4-day survival index and lactation index were reduced in litters at 150 mg/kg bw/day. At ≥ 50 mg/kg bw/ day the body weight of male weanlings were significantly reduced on day 21 post partum.	Males: NOAEL = 50 mg/kg bw/day LOAEL = 150 mg/kg bw/day Females: not affected NOAEL ≥150 mg/kg bw/day (highest dose)	LOAEL = 50 mg/kg bw/day (lowest dose)	n.a.	Confidential, 1973a* A_6_8_2(1)
Oral, gavage	Perinatal+ lactation study No guideline Non-GLP	Rat Albino 17-19 ♀ per group	From Day 15 of gestation through-out gestation and lactation	0, 50, 150 mg/kg bw/day	At ≥ 50 mg/kg bw/day both the 12-day survival index and the 21-day lactation index were reduced.	NOAEL = 150 mg/kg/day (highest dose)	LOAEL = 50 mg/kg bw/day (lowest dose)	n.a.	Confidential, 1973b* A6_8_2(2)

***** The studies were found to be of limited quality and not fulfilling the data requirement for biocides.

Route of exposure	Test type, Method, Guideline	Species, Strain, Sex, no/group	Exposure Period	Doses	Critical effect	NOAEL/ LOAEL Parental	NOAEL/ LOAEL F1	NOAEL F2	Reference
Oral, gavage	Two-generation study OECD 416	Rat Wistar (HsdCpb: WU conventionally bred) 30/ sex/group	♂: 10 weeks pre-mating, 2 wks mating period ♀: 10 weeks pre-mating, through-out gestation and lactation	0, 60, 180, 540 mg/kg bw/day	A significant and dose-related decrease in terminal body weight was observed in P males at ≥ 180 mg/kg bw/day and in F1 males at ≥ 60 mg/kg bw/day. Reduction in body weight gain during gestation was observed in dams at 540 mg/kg bw/day in the P generation and at 180 and 540 mg/kg bw/day in the F1 generation. Treatment-related kidney effects (nephropathy, dilated tubules, basophilic tubules and lymphocytic infiltration) were observed in P and F1 males at ≥ 60 mg/kg bw/day and P and F1 females at 540 mg/kg bw/day. A significantly lower female fertility index was observed at 540 mg/kg bw/day in the P generation and at 180 and 540 mg/kg bw/day in the F1 generation. A significantly longer oestrous cycle length and reduced fecundity was observed at 540 mg/kg bw/day in the F1 dams compared to control animals. **(Repr Cat 2 H361f: Suspected of damaging fertility)**Pups: Decreased terminal body weights at ≥ 180 mg/kg bw/day in the F1 litter and at 540 mg/kg bw/day in the F2 litter. Lower percentage of ear and eye opening at 540 mg/kg bw/day and incisor eruption at 180 mg/kg bw/day was found in both generations.	Males: LOAEL = 60 mg/kg bw/day (lowest dose)Females: NOAEL= 180 mg/kg bw/day LAOEL= 540 mg/kg bw/day	Parental F1: Males: LOAEL = 60 mg/kg bw/day (lowest dose) Females: NOAEL = 60 mg/kg bw/day LOAEL = 180 mg/kg bw/day Pups F1: NOAEL = 60 mg/kg bw/day LOAEL = 180 mg/kg bw/day	NOAEL = 60 mg/kg bw/day LOAEL = 180 mg/kg bw/day	Confidential, 2008 A6_8_2(3) **KEY STUDY**

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There are submitted two studies examining **fertility and sexual function** and one follow up study on **lactation, all in rats** . The one-generation study and the perinatal and lactation study are both non-GLP and performed before guideline. Individual data are missing and the purity of the test compound is not given. The studies were found to be of limited quality and not fulfilling the data requirement for biocides (reproductive toxicity), hence a two-generation study was required in the completeness check phase of the evaluation. The two-generation study submitted by the applicant follows the OECD 416 guideline, are GLP and the purity and stability of the test compound are given.

The **one-generation** study was performed in albino **rats.** In this study male body weight gain was reduced in the high-dose group (150 mg/kg/bw/day) during the pre-mating period. The body weight reduction continued until the end of the study. Females of either dose group were not affected. There were no effects on the number of implantation sites, resorption sites and corpora lutea. Reproductive performance was not affected in any treatment groups. All delivered pups were normal in appearance. A dose dependent reduced 4-day survival and lactation index (79,1 and 61,1 % in the 50- and 150-mg/kg bw/day groups, compared with the control value of 80,4 mg/kg bw/day) was observed. Body weights of male weanlings in the 50- and 150-mg/kg bw/day groups were significantly lower than in control weanlings. Hence, the parental NOAEL in this study is set to 50 mg/kg bw/day for the males based on the reduced body weight. Since there were no effects observed in the dams the maternal NOAEL is ≥150 mg/kg bw/day The LOAEL for the F1 generation pups is 50 mg/kg bw/day based on the reduced body weight in the male weanlings. The maternal NOAEL in this study is higher than the NOAEL for the weanlings and it can not be excluded that the effect observed are treatment related. However, since there are shortcomings in the study design and report like; the reduced body weight in the male weanlings are not dose-dependent and individual data are not provided, the findings can not be used for classification of developmental toxicity.

A **two-generation reproductive toxicity study** (OECD 416) was recently conducted in **Wistar rats**. This oral gavage study (doses: 60, 180, 540 mg/kg/day) confirmed that the kidney is the target organ for general chlorophene toxicity in rats (please refer table 29 below for further details). Increases in absolute and relative kidney weights were observed in all treated males of both generations (please refer to table 29 for details). These findings were associated with macro- and microscopic renal lesions. A reduction in body weight gain during gestation was observed in dams at 540 mg/kg bw/day in the P generation and at 180 and 540 mg/kg bw/day in the F1 generation (please refer to table 4.13 for details). Body weights of the F1 pups were reduced at 180 and 540 mg/kg bw/day at lactation day 7, 14 and 21 (please refer to table 4.13 for details). The maternal body weight was unaffected at 60 and 180 mg/kg bw. No treatment-related changes in food intake were recorded in the dams. In the F2 pups body weights were reduced during lactation in the 540-mg/kg bw/day group. At the same dose level some effects on maternal bodyweight were recorded on lactation day 1. Lower percentage of ear and eye opening at 540 mg/kg bw/day and incisor eruption at 180 mg/kg bw/day was observed in both F1and F2 pups.

In this study effects on fertility, fecundity and oestrus cycle length were reported. A significantly lower female fertility index was observed in the P generation at 540 mg/kg bw/day and at 180 and 540 mg/kg bw/day in the F1 generation, and a reduced fecundity was observed at 540 mg/kg bw/day in the F1 generation. A significant increase in oestrus cycle length (4.5 days) was observed in the F1 females after treatment with 540 mg/kg bw/day. In the study report this effect is suggested

to be incidental since the observed oestrus cycle length is within the historical control range (4.3-4.5). However, the oestrus cycle length of the concurrent control animals as well at the animals in the two lowest dose groups are lower than the historical control range (4.1, 4.0 and 4.1, respectively), and hence the effect should be compared to the experimental control group rather than the historical control range. Based on these findings chlorophene has an effect on the oestrus cycle length.

The overall NOAEL for adverse effect on development of the offspring is **60 mg/kg bw/day** based on the reduction in body weight in the F1 pups at 180 mg/kg bw/day. The NOAEL for adverse effects on sexual function and fertility is **60 mg/kg bw/day** based on the reduced female fertility index observed in the F1 generation. The **parental LOAEL** is **60 mg/kg bw/day** based on the kidney effects of male rats of both generations.

Table 29: Table for two-generation reproductive toxicity study in rats (confidential, 2008 / A6_8_2(3))

Parameter			Dose [mg/kg bw/day]								Dose response +/–	
			0		60		180		540			
		Generation	m	f	m	f	m	f	m	f	m	f
Food consumption		**P**	–	–	–	–	–	–	–	↑	–	+
		F1	–	–	–	–	–	–	–	↑	–	+
Body weight gain males: pre-mating females: gestation	% of control	**P**	100	100	96	101	91	94	72*	71*	+	+
		F1	100	100	94*	99	92*	86*	82*	81*	+	+
Organ weights												
Kidney	abs [g]	**P**	2.531	1.808	2.764*	1.817	2.735*	1.900	5.076*	2.401*	+	+
		F1	2.397	1.807	2.534*	1.888*	2.567*	1.832	3.020*	2.302*	+	+
	rel [% bw]	**P**	0.553	0.699	0.613*	0.706	0.640*	0.727	1.342*	0.911*	+	+
		F1	0.548	0.667	0.609*	0.693*	0.647*	0.688	0.839*	0.870*	+	+
Adrenals	rel [% bw]	**P**	0.013	0.033	0.014*	0.034	0.014*	0.034	0.018*	0.035	+	–
		F1	0.014	0.031	0.014	0.033*	0.016*	0.032	0.017*	0.033	+	–
Pathology												
Kidney, surface uneven or rough	Incidence	**P**	0	0	0	0	0	1	30	3	+	+
		F1	0	0	0	0	0	0	17	5	+	+
Histopathologic examination												
Kidney, lymphocyte infiltration	Incidence	**P**	0	0	0	2	2	1	0	8	–	+
		F1	2	0	0	0	0	0	0	2	–	–
Kidney, dilated tubules or nephropathy	Incidence	**P**	0	0	1	0	14	1	30	22	+	+
		F1	0	0	2	0	11	1	30	18	+	+
Adrenals, hypertrophy zona glomerulosa	Incidence	**P**	0	0	/	1	/	0	3	2	±	–
		F1	0	2	0	0	0	0	12	4	+	–
Oestrus cycle length, prior to co-habitation	[d]	**P**	–	4.5	–	4.2	–	4.5	–	4.5	./.	–
		F1	–	4.1	–	4.0	–	4.1	–	4.5*	./.	–
Reproductive Performance												
Fertility index		**P**	93.3		86.7		86.7		76.7*		+	
		F1	100.0		100.0		90.0*		83.3*		+	
Fecundity index		**P**	96.4		96.2		96.2		95.7		–	
		F1	100.0		100.0		96.3		96.0*		+	
Pup effects												
Pup weight PND1	[g]	**F1**	5.9		6.1		5.9		5.4*		+	
		F2	5.9		5.9		5.8		5.5*		+	
Pup weight PND21	[g]	**F1**	46.1		45.3		43.0*		39.1*		+	
		F2	44.3		44.5		43.6		40.5*			
Incisors erupted	% on PND11	**F1**	84.9		75.8		67.0*		45.5*		+	
		F2	89.5		87.0		72.4*		62.3*		+	
Ears open	% on PND14	**F1**	78.6		72.4		84.8		28.6*		+	
		F2	96.5		96.7		97.9		34.9*		+	

Parameter			Dose [mg/kg bw/day]								Dose response +/–	
			0		60		180		540			
		Generation	m	f	m	f	m	f	m	f	m	f
Eyes open	% on PND16	F1	100.0		98.4		100.0		91.6*		+	
		F2	99.6		98.6		99.0		97.7		–	
* Statistically significant difference to controls, p<0.05												

4.9.1.1 Summary and discussion of effects on sexual function and fertility

In the one-generation study in rat, male body weight gains were reduced in the high-dose group during the pre-mating period. Females of either dose group were not affected. There were no effects on the number of implantation sites, resorption sites and corpora lutea. Reproductive performance was not affected in any treatment groups. All delivered pups were normal in appearance. Based on reduced body weight the **paternal NOAEL is 50 mg/kg bw/day**. Since there were not reported any maternal treatments related effects, **maternal NOAEL is 150 mg/kg bw/day** (highest dose). Based on reduced 4-day survival and 12-day survival and lactation index the **offspring LOAEL is 50 mg/kg bw/day** (lowest dose). Due to insufficiencies in the study design of both the one-generation and lactation study, the two-generation study is put as the key study for **fertility**. The **two-generation reproduction** oral gavage study in rats is recently performed (2008) and it confirmed that the kidneys are the target organ of chlorophene in rats. A reduction of body weight gain during gestation was observed in dams of both generations at 540 mg/kg bw/day and at 180 mg/kg bw/day. Pup weights of the F1 and F2 generation were reduced in the 180- and 540-mg/kg groups, respectively. A significantly lower female fertility index was observed in the P generation at 540 mg/kg bw/day and at 180 and 540 mg/kg bw/day in the F1 generation. A significantly increased oesterous cycle length and reduced fecundity was observed at 540 mg/kg bw/day in the F1 dams. It is likely that the reduced female fertility index is treatment-related as it was observed in both generations. Based on this the **NOAEL for adverse effects on sexual function and fertility** should be **60 mg/kg bw/day**. The overall **NOAEL for adverse effect on development of the offspring** is **60 mg/kg/day** based on the reduction of F1 pup weights in the two-generation study at 180 mg/kg bw/day. The **parental LOAEL** is **60 mg/kg bw/day** based on the kidney effects of male rats of both generations.

4.9.1.2 Comparison with the CLP criteria

The effect on fertility, fecundity and oestrus cycle length were found at dose levels were some effects (reduced bw gain) of chlorophene were observed. In the Guidance on the application of the CLP Criteria (2009) it is stated in "*3.7.2.2.1.1. Effects to be considered in the presence of marked systemic effects" that adverse effect on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes".* In this study the reduced maternal bw gain at the highest dose in P and F1 generation were lower than 10% compared to the control group. No lethality or coma related to treatment was observed at any dose levels. Further in the guidance it is stated "*There is no established relationship between fertility effects and less marked systemic*

toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity." Since the female fertility index was significantly reduced in both generations the effect is likely to be treatment-related. In addition, the fecundity and oestrous cycle was affected in the F1 dams of the highest dose-group. Based on the effects on fertility, fecundity and oestrus cycle length, chlorophene should be classified for fertility.

4.9.1.3 Conclusions on classification and labelling

Based on the reduced female fertility index observed in both generations and reduced fecundity and increased oesterous cycle length in the two-generation reproductive study, chlorophene should be classified for fertility effects. **A classification for fertility is proposed:**

Repr Cat 2 H361f: Suspected of damaging fertility according to Regulation (EC) No 1272/2008 (CLP).

4.9.1.4 Summary and discussion of effects on lactation

A dose dependent reduced 4-day survival and lactation index was observed in the one generation study performed in **albino rats**. A dose-dependent reduction in lactation index was observed, but no cross fostering was performed. No information on the purity and stability of the test material was provided.

A follow-up study (confidential, 1973b / A6_8_2(2)) was performed (table 30) to examine the effects of chlorophene on prenatal and postnatal performance of albino rats. Female rats were treated with 50 or 150 mg/kg bw/day from day 15 of gestation throughout the lactation period. Body weights of test and control females revealed no differences which could be attributed to the exposure to chlorophene. There were no deaths or abnormal behavioural reactions observed during the investigation. The number of pups delivered viable and retained through lactation day 4 were similar for all groups. Dose-dependent reduction in pups retained by females exposed to chlorophene compared to control females was observed on lactation days 12 and 21 (weaning) in the 50 and 150 mg/kg bw/day groups.

Table 30: Perinatal and lactation study in rats

Parameter	Controls	50 mg/kg	150 mg/kg	Dose-response
4-day survival index $\frac{\text{Number of Pups Viable at Lactation Day 4}}{\text{Number of Viable Pups Born}} \times 100$	98.6	96.7	97.9	–
12-day survival index $\frac{\text{Number of Pups Viable at Lactation Day 12}}{\text{Number of Pups Retained at Lactation Day 4}} \times 100$	97.9	88.9	86.9	–
Lactation index $\frac{\text{Number of Pups Viable at Lactation Day 21}}{\text{Number of Pups Retained on Lactation Day 4}} \times 100$	90.5	77.2	77.0	–

4.9.1.5 Comparison with the CLP criteria and conclusions on classification and labelling

Based on insufficiencies in the first study design (confidential, 1973a / A6_8_2(1)) these finding does not support classification for lactation.

It can not be excluded that the reductions in survival indexes a in the second study (confidential, 1973b / A6_8_2(2)) are treatment-related, but since no individual animal data was provided, no cross fostering test was performed and the effects observed were not dose dependent, no classification for lactation is recommended.

4.9.2 Developmental toxicity

Table 31: Teratogenicity of chlorophene

Route of exposure	**Test type, Method, Guideline**	**Species, Strain, no/group**	**Exposure Period**	**Doses**	**Critical effects** **1) dams** **2) foetuses**	**NOAEL / LOAEL Maternal toxicity**	**NOAEL / LOAEL Teratogenicity, Embryo-toxicity,**	**Reference**
Oral, gavage	Developmental toxicity No guideline, but ≅ OECD 414	Rat CD (Sprague-Dawley derived) ♀ 20 /group	Day 6-15 of gestation	0, 100, 300, 900 mg/kg bw/ day	1) At 300 mg/kg bw/day and 900 mg/kg bw/day animals showed significantly reduced body weight and food intake. 2) No treatment-related effects on litter size or foetal survival, weight and morphological development.	NOAEL = 100 mg/kg bw/day LOAEL = 300 mg/kg bw/day	NOAEL = 900 mg/kg bw/day (highest dose)	Confidential, 1985a A6_8_1(1)
Oral, gavage	Developmental toxicity Dose-range finding study Non-GLP	Rat CD (Sprague-Dawley derived) ♀, 6 /group	Day 6-15 of gestation	0, 100, 300, 1000 mg/kg bw/ day	1) At ≥ 100 mg/kg bw/day the overall weight gain was significantly reduced (day 6-15). One animal at 1000 mg/kg bw died after rapid weight and condition loss. 2) At 1000 mg/kg bw/day: foetal weight was reduced	LOAEL = 100 mg/kg bw/day (lowest dose)	NOAEL = 300 mg/kg bw/day LOAEL = 1000 mg/kg bw/day	Confidential, 1985b A6_8_1(4)

Route of exposure	**Test type, Method, Guideline**	**Species, Strain, no/group**	**Exposure Period**	**Doses**	**Critical effects** **1) dams** **2) foetuses**	**NOAEL / LOAEL Maternal toxicity**	**NOAEL / LOAEL Teratogenicity, Embryo-toxicity,**	**Reference**
Oral, gavage	Developmental Toxicity No guideline, but ≅ OECD 414	Rat Wistar (KFM-HAN) ♀ 25 /group	Day 6-15 of gestation	0, 15, 75, 375 mg/kg bw/day	1) At 375 mg/kg bw/day reduced body weight gain and food intake was observed. Three females of the 375 mg/kg bw/day group died during the treatment period. 2) The body weight was slightly but significantly reduced in the foetuses of the 375 mg/kg bw/day group and an increased incidence of non-ossified phalangeal nuclei was observed.	NOAEL = 75 mg/kg bw/day LOAEL = 375 mg/kg bw/day	NOAEL = 75 mg/kg bw/day LOAEL = 375 mg/kg bw/day	Confidential, 1984 A6_8_1(3) **KEY STUDY**
Oral, gavage	Developmental toxicity No guideline, but ≅ OECD 414	Rabbit NZW ♀ 14-16 /group	Day 6-19 of gestation	0, 10, 30, 100 mg/kg bw/ day	1) No effects on treated dams. 2) No adverse effects on foetuses.	NOAEL =100 mg/kg bw/day (highest dose)	NOAEL =100 mg/kg bw/day (highest dose)	Confidential, 1985c A6_8_1(2) **KEY STUDY**
Oral, gavage	Developmental toxicity Dose range finding study Non-GLP	Rabbit NZW ♀, 4/group	Day 6-19 of gestation	0, 30, 100, 150, 200, 300 mg/kg bw/ day	1) High mortality at 200 and 300 mg/kg bw. At 150 mg/kg/bw two animals was killed in extremis and abortions related to reduced bw was observed. 2) No compound related effects were observed at any dose-level.	NOAEL=100 mg/kg bw LOAEL=150 mg/kg bw	NOAEL = 150 mg/kg bw/day (highest dose)	Confidential, 1985d A6_8_1 (4)
Oral, gavage	Developmental toxicity No guideline, but ≅ OECD 414 Non-GLP	Rabbit NZW ♀ 24 /group	Days 7-19 of gestation	0, 40, 80, 160 mg/kg bw/day	1) At 160 mg/kg bw/day body weights of dams were reduced during treatment, in addition a total of 10 of 24	NOAEL = 80 mg/kg bw/day (mortality, impaired bw gain) LOAEL = 160 mg/kg	NOAEL = 80 mg/kg bw/day. LOAEL = 160 mg/kg bw/day;	Confidential, 1979 A6_8_1(5)

Route of exposure	Test type, Method, Guideline	Species, Strain, no/group	Exposure Period	Doses	Critical effects 1) dams 2) foetuses	NOAEL / LOAEL Maternal toxicity	NOAEL / LOAEL Teratogenicity, Embryo-toxicity,	Reference
					(approximately 42 %) females died. 2) None of the examined effects observed in the foetuses were considered to be treatment-related. Due to high maternal mortality in the highest dose group a proper embryotoxic/teratogenic evaluation could not be performed.	bw/day.	no clear teratogenic or embryotoxic effects, but the maternal lethality was too high that one could conclude upon possible effect on the foetuses at this dose.	

Three oral **developmental toxicity** studies were performed in the **rat**. In these studies pregnant female rats were exposed to chlorophene by gavage from day 6 to 15 of gestation. In the key study (confidential, 1984 / A6_8_1(3)) reduced body weight gain and food intake was observed in the dams at 375 mg/kg bw/day. The body weight was reduced in the foetuses of the 375 mg/kg bw/day group and an increased incidence of non-ossified phalangeal nuclei was observed. This effect was probably secondary to the weight depression. The **NOAELs for maternal toxicity and developmental toxicity in rats** are both **75 mg/kg bw/day.** A limitation to these teratogenic studies in rats is that the dams have only been exposed to chlorophene during organogenesis and not from implantation and all the way through the gestation as required in the current version of OECD guideline 414 (2001).

The three **developmental toxicity** studies in **rabbits** did not reveal any clear adverse effects on foetal development. In the key study (confidential, 1985c / A6_8-1(2)) no adverse effect on dams or the foetuses were observed at highest dose level (100 mg/kg bw/day). In a dose range finding study (confidential, 1985d /A6_8_1 (4) no treatments related effects were found at 100 mg/kg bw/day (NOAEL) in dams or at 150 mg/kg bw/day in foetuses (NOAEL). High mortality (2 of 4 animals died) of the dams was observed at 150 mg/kg bw/day, hence 100 mg/kg bw/day was used as highest dose level in the main study. Since the MTD seems to be between 100 and 150 mg/kg bw/day, a slightly higher top dose in the main study would have been preferable in the main study (confidential, 1985c / A6_8-1(2)).

In an older study (confidential, 1979 A6_8-1(5)) the mortality rate was significantly higher than control at the high-dose level (160 mg/kg bw/day) where 10 of 24 high-dose females died. According to OECD guideline 414, each test and control group should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy. Groups with fewer than approximately 16 animals with implantation sites may be inappropriate. Maternal mortality does not necessarily invalidate the study providing it does not exceed 10 %. In the study the mortality was approximately 42 %. Therefore, the LOAEL of embryotoxic/teratogenic effects of 160 mg/kg bw/day could not be justified due to high maternal mortality. In addition, due to several shortcomings in the study design (uncertainties concerning purity and stability of the test substance, no information whether females inseminated by the same male were evenly distributed across the groups, maternal organ weights like the gravid uteri including the cervix were omitted and the heads of one-half of the foetuses examined should have been removed and processed for evaluation of soft tissue alterations in the brain, and this was also omitted) the assessment of maternal and offspring toxicity was incomplete. However based on the results given in the study a NOAEL of 80 mg/kg bw/day for maternal and embryotoxic/teratogenic effects was concluded..

Similarly to the studies on rats, the rabbits were only exposed to chlorophene during the period of organogenesis (days 6/7-19) and not all the way from implantation and through gestation. Based on the key study (confidential, 1985c / A6_8-1(2)) **NOAELs for maternal toxicity and developmental toxicity in rabbits** are both **100 mg/kg bw/day.**

4.9.2.1 Summary and discussion of developmental toxicity

Summary and discussion on developmental toxicity

The **developmental toxicity** studies in **rabbits** did not reveal any adverse effects on foetal development. Maternal and foetal body weight gain was the only affected parameter in the

developmental toxicity studies in **rats**. The **NOAEL rabbits for maternal effects is 100 mg/kg** bw/day and the **NOAEL rabbits for developmental toxicity is 100 mg/ kg bw/day (highest dose tested).** The **NOAELs for maternal toxicity and developmental toxicity in rats** are **75 mg/kg bw/day**.

4.9.2.2 Conclusions on classification and labelling

No classification is proposed.

RAC evaluation of reproductive toxicity

Fertility and reproductive function

Summary of the Dossier submitter's proposal

Data were available from a 2-generation reproductive toxicity study (OECD TG 416) in the rat (from 2008) and two older non-guideline studies: a 1-generation study and a perinatal/lactation study, also both conducted in the rat (from 1973). The 2-generation study had been required in the completeness check phase of the formal evaluation of chlorophene as a biocide because both the 1-generation study and the perinatal/lactation study were considered to be unreliable and not fulfilling the data requirement for biocides.

In the 2-generation study, rats were dosed by gavage at dose levels of 0, 60, 180 and 540 mg/kg bw/day. A reduction of parental body weight was observed at 180 and 540 mg/kg bw/day and pup body weight was reduced in the F1 and F2 generations at 540 mg/kg bw/day and F1 generation only at 180 mg/kg bw/day. A significantly lower female fertility index was observed in the P generation at 540 mg/kg bw/day and in the F1 generation at 180 and 540 mg/kg bw/day. Oestrous cycle length was found to be significantly increased in F1 dams at 540 mg/kg bw/day and reduced fecundity was also observed in F1 dams at this dose. There were no effects on postnatal survival at any dose tested.

In the non-guideline, non-GLP, 1-generation study, rats received a dose of 0, 50 or 150 mg/kg bw/day by gavage. Male body weights were reduced in the parental generation, but female body weights remained unaffected. There were no effects on fertility or reproductive function in this unreliable study. The perinatal/lactation study did not provide any useful information for the DS in the context of their assessment of the effects of chlorophene on fertility and reproductive function.

Whilst the effects on fertility index, fecundity and oestrous cycle seen in the 2-generation study occurred at doses also causing maternal toxicity (reduced body weight gain), there was no established relationship between fertility effects and less marked systemic toxicity. Therefore it was assumed by the DS that effects on fertility seen at dose levels causing less marked systemic toxicity were not a secondary consequence of this toxicity. The reduced maternal body weight gains at the highest doses in the P and F1 generations were lower than 10% compared to the control groups. No lethality related to treatment was observed at any dose level. Since the female fertility index was statistically significantly reduced in both generations the effect was likely to be treatment–related. In addition, the fecundity and oestrus cycle were affected in the F1 dams of the highest dose-group. Based on the effects on fertility, fecundity and oestrus cycle length, and with reference to the CLP criteria, para. 3.7.2.2.1.1 (2009), the DS proposed to classify chlorophene as Repr. 2 for fertility (H361f).

Comments received during public consultation

There were four comments submitted regarding fertility during the public consultation. One MSCA agreed with the proposed classification for health hazards in general.

One MSCA was in agreement with classification as Repr. 2 (H361f), specifically based on reduced female fertility index. The MSCA commented that it was unclear whether the reduced fertility was secondary to maternal toxicity. They added a comment about an additional study not described in the CLH report showing chlorophene binding to the androgen receptor and, without any further explanation, that this occurred at a similar level of "potency" to the "CYP inhibition findings." This MSCA further commented that both androgen receptor binding and CYP inhibition are associated with delayed sexual development and decreases in reproductive performance.

In response, the DS provided more details of the receptor binding study. Apparently this was part of the US EPA's ToxCast research programme, which uses high throughput screening to profile bioactivity and for predicting the toxicity of large numbers of chemicals. Chlorophene was included in Phase 1 of the programme. Based on *in vitro* testing, chloroprene was found to bind the androgen receptor and to inhibit CYP enzymes at similar potencies, which were both associated with delays in sexual development and decrements in reproductive performance. Chlorophene was identified by the authors as a predicted reproductive toxicant.

A second MSCA sought clarification on how the classification for fertility was derived. The MSCA required a thorough analysis of the data, in particular, individual animal data for fertility index and body weight gain, historical control data and data relating to males, e.g. spermatogenesis. The MSCA noted that the maternal body weight gain was reduced by up to 30% in the top dose group and suggested that the increased oestrous cycle length, the reduced fertility index and fecundity index could all be secondary to this effect. In response, the DS clarified that the reduced body weight gain in exposed groups was in fact less than 12% compared to the control group during gestation. This is shown in the Table in "Additional key elements" section in the BD).

A manufacturer of chlorophene argued for no reproductive toxicity classification. They doubted the relevance of the magnitude of the responses observed in the 2-generation study and considered that the effects on fertility were secondary to maternal toxicity. They provided historical control data from the laboratory where the study was conducted with the suggestion that the effects on fertility index, fecundity and oestrous cycle length were due to biological variability rather than due to treatment with chlorophene.

The manufacturer queried the cause of the reduced fertility in top dose female rats, noting it could be incidental rather than treatment-related as there were no other effects on related parameters such as oestrous cyclicity or gross or microscopic findings of the reproductive organs in these animals. The manufacturer attributed the changes in fertility in female rats as secondary to maternal toxicity. They described how female weight gain was reduced by up to 30% during gestation* in the top dose group and that findings in the kidney at termination were suggestive of nephrotoxicity (See table in the "additional key elements" section in the BD for corrected bw gains). However, RAC notes the response of the DS on the same point to the second MS (described above). Historical control data showed female fertility indices ranging from 88 – 100% for the P generation. The manufacturer deemed the values of 77 and 83% in the 2-generation study to be borderline responses occurring in the presence of maternal toxicity. RAC notes after independent evaluation of the laboratory historical control data that errors had been made in the calculation for fertility index HCD. The correct range was 80-100%.

The lowered fecundity index of 96.0% in the F1 females was statistically significant. Historical control data was provided by the manufacturer that gave a range of 86.7 –

100% for this type of study in this laboratory. Therefore, the manufacturer deemed this finding incidental rather than treatment-related.

The manufacturer also commented on the observed increased oestrous cycle duration seen in F1 females given the top dose. The value of 4.5 days was found to be statistically significantly increased compared to the concurrent F1 control (4.1 days), but an oestrus cycle of 4.5 days was also seen in the P1 control, mid and high dose groups. It was therefore suggested that the oestrous cycle differences were likely to be attributed to biological variability rather than to treatment with chlorophene.

Additional key elements

In responding to the public comments, the DS provided corrected values of maternal body weight gain during gestation and also information on body weight gain in the pre-mating exposure period. The corrected body weight gains for the gestation period are presented in the table below alongside the relevant fertility findings in female P and F1 rats of the 2-generation study. Prior to mating (weeks 0 – 10), there were no statistically significant differences in body weights and net weight gain between the vehicle control groups and all the treated groups.

Table. Summary of relevant parameters on female rats dosed with chlorophene in a 2-generation study

Parameter	**Dose (mg/kg bw/day)**				**Historical Control Data (%)**
	0	60	180	540	2002-2012
Net body weight gain (pre-mating period)					
P	80 g	88 g	85 g	83 g	
% Body weight gain (gestation)					
P	0	-1	-3	-12	
F1	0	-1	-5	-7	
Fertility Index†					
P	93.3	86.7	86.7	76.7*	80-100**
F1	100.0	100.0	90.0*	83.3*	80-100**
Fecundity Index					
P	96.4	96.2	96.2	95.7	**
F1	100.0	100.0	96.3	96.0*	**
Oestrous cycle length					
P	4.5	4.2	4.5	4.5	**
F1	4.1	4.0	4.1	4.5*	**

(*) Statistically significant difference from controls, $p<0.05$

(**) Historical control data were provided from the testing laboratory for 9 studies, the first of which commenced in 2002 and the last of which ended in 2011. The study with Chlorophene ended in 2008. For discussion on the historical control data, refer to the text below.

The analysis of historical control data made by the testing laboratory was flawed. Fertility (%) was described in the chlorophene study report as being the number of pregnant females (confirmed at necropsy)/number of sperm positive females)x100. When RAC calculated the fertility values for each historical study, the results did not match those cited by the manufacturer. The testing laboratory defined fecundity (%) as being the number of female rats with at least one viable foetus/the number of pregnant animals. Although the company provided historical control values, it was not clear which data were used to derive them and the values given seemed to be at odds with data presented. For example, for a study conducted in 2002-2003, with group size 30 animals, there were 28 live litters recorded from 28 pregnant females. The company stated the fertility in this study as 100% and fecundity was 93.33%. Given their definitions of fertility and fecundity, these values were erroneous. As a result, RAC took a cautious approach when interpreting the analysis of the data by the testing laboratory. The historical control values for fertility given in the

table were calculated by RAC from the data provided. It was not possible to calculate fecundity values as figures for the number of female rats with at least one viable foetus were not provided. No historical data were given for oestrus cycle length.

Assessment and comparison with the classification criteria

In a 1-generation study (1973), no effects on fertility were observed. As commented by the DS, this study was inadequate for assessing the effects of chloroprene on fertility and reproductive function. Therefore, the focus of RAC's assessment is the more recent, guideline and GLP compliant, 2-generation study performed in Wistar rats.

Wistar rats were given chlorophene by gavage, males for 10 weeks prior to mating and then 2 weeks during the mating period and females for 10 weeks prior to mating and throughout the gestation and lactation periods. Doses given were 0, 60, 180 and 540 mg/kg bw/day.

Effects on systemic toxicity:
P generation males suffered reduced terminal body weights at doses ≥ 180 mg/kg bw/day and reduced body weight gain at 540 mg/kg bw/day (-29%). Treatment-related kidney effects (nephropathy, dilated tubules, basophilic tubules and infiltration) were observed in P males at ≥ 60 mg/kg bw/day. The incidence and severity of these effects was higher in males when compared to females. No description of severity was noted; however the findings were consistent with those of a 95-day repeated dose toxicity study in rats in which the observed nephrotoxicity was described as minimal to mild (at doses < 240 mg/kg bw/day) and mild to moderate (at doses of 240 and 480 mg/kg bw/day).

Top dose treated P generation females had reduced body weight gain during gestation (-12%) the magnitude of which was much less at the mid-dose of 180 mg/kg bw/day (-3%). Importantly, there was no reduction in body weight in any group during the period prior of fertilisation and gestation (Table under "Additional key elements" in the BD). Similarly to males, kidney toxicity was reported, but only in the top dose group. Again, the severity of the nephrotoxicity was not reported, however the study report specified that females were found to be less sensitive to chlorophene than males.

F1 generation males had reduced terminal body weights at ≥ 60 mg/kg bw/day. Reduced body weight gain was also observed at doses ≥ 180 mg/kg bw/day. As with P males, kidney toxicity was noted at doses ≥ 60 mg/kg bw/day. F1 females had a small decrease in body weight gain at the top dose (-7%). Kidney toxicity was also observed at this dose level.

There were no reports of death, moribundity or significant toxicity in males or females of the P, F1 and F2 generations.

Effects on Fertility:
The fertility index was defined as the number of pregnant females (confirmed at necropsy)/number of sperm positive females. In the P generation, this was decreased from 93.3% in controls to 76.7% in the top dose group. Historical control data provided by the laboratory that conducted the study showed that the range previously observed in similar studies was 80-100%. A statistically significant decrease in fertility index was also observed in the F1 generation at the mid and high doses (90% and 83.3% at 180 and 540 mg/kg bw/day respectively versus 100% in controls). Historical control data for F1 females was also in the range of 80-100%. RAC considers the comparison with the concurrent control to be the most informative and on this basis there was a weak effect on fertility in this study. Given that the reduction seen in top dose P1 females was outside the historical control range, the historical control data do not contradict this conclusion.

Fecundity index was defined as the number of female rats with at least one viable

foetus/number of pregnant females. This was reported as statistically significantly decreased for top dose F1 females (96% versus 100% in controls). Historical control data provided by the testing laboratory showed that the percentage range for this effect in similar studies was 86.7-100%. However, RAC noted that the historical data provided by the laboratory didn't seem to support this calculated range and insufficient data were provided for RAC to calculate the historical control values independently. However, it is unclear whether this effect was treatment-related or an incidental effect and no effect was reported in the P generation. The percentage of animals affected was small and well within the historical control range that had been provided. Overall, this finding was not considered supportive of classification for fertility effects by RAC.

The statistically significant increase in oestrous cycle duration observed in F1 females of the top dose group (4.5 days versus 4.1 days in controls) was not considered supportive of classification by RAC on account of similar values being observed in control P females and other dose groups.

RAC Conclusion:

In addition to the evaluation of the CLH report and the information received during public consultation, RAC has also considered the information provided in the 2-generation study report itself. In this study, the authors concluded that there were no adverse effects on reproduction or fertility. However, RAC notes that the reduction in fertility index was found to occur in a dose-dependent manner which was reproducible in both P and F1 generations. Historical control data were provided by the testing laboratory for 9 studies between the years 2002 – 2011. The range for historical control female rat fertility index was 80-100% and the value derived for P females at 540 mg/kg bw/day in the current study was outside of this (76.7%). RAC agrees that this value was not marked when compared to the historical observations, but considers the concurrent control values to provide the most relevant comparison. There was a clear reduction in both generations when compared to historical control data. RAC is of the opinion that the slightly reduced fertility index observed in P and F1 generation rats treated with chlorophene in the 2-generation study were indicative of a weak adverse effect on fertility.

Pre-mating body weight of females were unaffected by chlorophene treatment. Kidney toxicity, whilst not explicitly stated in this study, was not considered severe at similar doses in a 95-day study in rats. A decrease in body weight gain (-12% at 540 mg/kg bw/day) occurred only during the gestation period and so was not considered relevant to the period during which fertilisation may be affected. As stated by the DS, there is no established relationship between fertility effects and less marked systemic toxicity. Therefore, it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity were not a secondary consequence of this toxicity.

RAC therefore concludes that classification for effects on fertility is warranted. As there is no human evidence to suggest that chlorophene is a known reproductive toxicant, category 1A is not appropriate. In consideration of category 1B, it is noted that the effect was weak and only observed in females, i.e. there was no evidence of testicular toxicity or other relevant effects in males. There were no changes to other reproductive parameters, no gross or microscopic findings to the reproductive system and litter sizes also remained unaffected. There is no indication of a mechanistic explanation for the effect observed on fertility. No effects on fertility were observed in a less robust 1-generation study. Taking all this into account the strength of evidence appears too weak to require a classification as Repr. 1B.

On the basis of dose-related changes to fertility index observed in female rats treated with chlorophene, occurring in the absence of marked systemic toxicity and to an extent that was outside of the relevant historical control range, **RAC therefore agrees with**

the DS that chlorophene should be classified Repr. 2 (H361f – suspected of damaging fertility).

Developmental toxicity

Summary of the Dossier submitter's proposal

There were three oral studies in rats and three in rabbits included in the CLH report for this endpoint, one of which in each species was a dose range finding study.

Each of the rat studies involved treatment by gavage with chlorophene from days 6 to 15 of gestation. A limitation of these teratogenic studies was therefore that the dams had only been exposed to chlorophene during organogenesis and not from implantation through birth. In what was described as a key study, reduced body weight and food intake were observed in the dams at the highest dose of 375 mg/kg bw/day. Foetal body weight was reduced in the foetuses of this dose group and an increased incidence of non-ossified phalangeal nuclei was seen. There were no observed adverse effects in dams or foetuses at the next lower dose of 75 mg/kg bw/day. The dose range finding study had previously shown that weight gain was reduced in dams at 100 mg/kg bw/day and foetuses at 1000 mg/kg bw/day. In the other rat study, reduced weight gain was seen in dams at 300 and 900 mg/kg bw/day; no treatment-related adverse effects had been seen in foetuses.

The rabbit studies did not reveal any clear adverse effects on foetal development. In what was described as a key study, no clear adverse effect was seen on dams or foetuses at the highest dose of 100 mg/kg bw/day. In a range-finding study, no treatment-related effects were found at 100 mg/kg bw/day in dams or at 150 mg/kg bw/day in foetuses. High mortality (2/4) had been evident in dams at 150 mg/kg bw/day, hence 100 mg/kg bw/day had been selected as the top dose in the main study. The DS commented that a slightly higher top dose would have been preferable, given that the MTD was between 100 and 150 mg/kg bw/day.

The DS was critical of the other rabbit developmental toxicity study. Mortality of dams (10/24) at the top dose of 160 mg/kg bw/day was significantly higher than in controls. This left less than the guideline number of dams with implantation sites at necropsy. The mortality rate of 42% exceeded the guideline-preferred limit of 10%. Therefore, the LOAEL of embryotoxic/teratogenic effects of 160 mg/kg bw/day was of limited value. The DS also noted several other shortcomings in study design (purity and stability of the test substance was uncertain, lack of information regarding the distribution of females inseminated by the same males, some maternal weights and other foetal measurements were omitted) that indicated the assessment of maternal and offspring toxicity was incomplete in this study.

No observed maternal of developmental adverse effects were seen in rabbits from 100 mg/kg bw/day. However, similarly to the studies in rats, the rabbits were only exposed to chlorophene during the period of organogenesis (days 6/7-19).

No classification for developmental toxicity was proposed by the DS.

Comments received during public consultation

No comments were received against the proposal for no classification. One MSCA described a developmental toxicity study in rabbits that had been summarised by the Californian Environmental Protection Agency (CEPA) (see below, under Additional key elements). This MSCA questioned whether this study was the same as one of those in the CLH report and whether the findings reported warranted classification of chlorophene for developmental toxicity. The manufacturer was supportive of the non-classification of

chlorophene for development.

In response to the comments made by this MSCA, the DS was not able to provide the study report within the given deadline. They acknowledged that the study had been evaluated by CEPA and that it was possible that there were adverse effects on development in this study following treatment with chlorophene.

Additional key elements

A brief summary of the results of a developmental/reproductive toxicity study in rabbits was made available during the public consultation. This study (Ross, 1992) had not been included in the CLH report. Chlorophene was given by gavage to groups of 14-21 mated new Zealand White rabbits on days 6-19 of gestation at 0 (corn oil), 10, 30 or 100 mg/kg bw/day. Findings apparently included an increased post-implantation loss and an increased incidence of ectopic kidney, ectopic testis and malformed kidney in foetuses at 100 mg/kg bw/day. No further details were provided.

Ref: Ross FW, 1992. Chloroprene: Effects of oral administration upon pregnancy in the rabbit. Supplement to LSR report #: 85/BTP033/257; LSR Ltd

Assessment and comparison with the classification criteria

The available studies in rats do not provide any findings to justify classification of chlorophene for developmental toxicity. Foetal body weight and an increased incidence of non-ossified phalangeal nuclei were evident at 375 mg/kg bw/day concomitant with reduced body weight and food intake in dams. In a second study, at comparable doses, no adverse effects were seen in foetuses. However, these studies only involved dosing during gestation days 6-15, so were limited in scope. In rabbits, as described by the DS, no clear adverse foetal effects were seen in the studies presented in the CLH report. However, as in rats, the dosing schedule was limited to the main period of organogenesis (gestation days 6/7 to 19). According to the DS, the additional data provided in the public consultation related to a further, more recent study in rabbits with a similar dosing schedule to the key study cited in the CLH report. In contrast to that study, this was summarised as showing increased post-implantation loss and an increased incidence of ectopic kidney, ectopic testis and malformed kidney in foetuses at 100 mg/kg bw/day. Unfortunately, no further details have been provided about the incidences and/or severity of these effects or about the maternal findings. In the absence of clearer, unambiguous information, given the contrast to the other studies, this is not viewed as sufficient evidence to support classification.

There were also relevant findings in the oral two-generation study conducted in rats (gavage, 0, 60, 180 and 540 mg/kg bw/day chlorophene). No overt signs of toxicity were seen in foetuses, providing further reassurance that no classification for pre-natal developmental toxicity is warranted. Pup body weights were reduced during the lactation period, measured on PND 1, 4, 7 ,14 and 21 at 540 mg/kg bw/day in both generations (e.g. mean pup weights at PND1 and PND 21 were 5.4/5.5 g and 39.1/40.5 g in F1/F2 pups, compared to 5.9/5.9 g and 46.1/44.3g in controls). These reductions appeared to be associated with reduced body weight gains of dams during the gestation period (by 20-30% and 5-15% compared to controls at 540 and 180 mg/kg, respectively). Similarly, at 540 mg/kg bw/day, there were lower percentages of incisor eruption (PND11), ear opening (PND14) and eye opening (PND16) in both generations. Decreased incisor eruptions were also evident at 180 mg/kg bw/day. RAC agrees with the interpretation of the DS that this slight delay in the acquisition of developmental landmarks was suggestive of an overall pattern of slight developmental delay in rats exposed to chlorophene. These observations correlated closely with the reduced body weight gains of pups and dams in the treated groups and **do not indicate a significant adverse effect**

on development warranting classification.

Lactation

Summary of the Dossier submitter's proposal

A non-guideline, non-GLP perinatal and lactation study was carried out in rats. The purpose of this study was to clarify possible effects on 4-day survival and lactation index observed in a 1-generation study. Female rats were treated with 0, 50 or 150 mg/kg bw/day chlorophene on day 15 of gestation through throughout the lactation period. The number of pups delivered viable and retained through lactation day 4 were similar across all groups. There was a reduction in survival index on lactation days 12 – 21 (weaning) in the 50 and 150 mg/kg bw/day groups. This study suffered a number of limitations and deficiencies. These deficiencies included a lack of individual animal data, that no cross-fostering test was carried out, and that the effects observed were not always dose-dependent. On that basis, the findings were not sufficient for classification for adverse effects on lactation.

Comments received during public consultation

There were no specific comments relating to effects of chlorophene on lactation.

Assessment and comparison with the classification criteria

Chlorophene was also tested for effects on lactation in rats. Pregnant females were treated with chlorophene (0, 50, 150 mg/kg bw/day) from day 15 of gestation onwards and throughout lactation. The results of the study showed there was reduced survival at 12 days; however this was not statistically significant. The survival index during lactation [(number of pups viable at lactation day 21/number of pups retained on lactation day 4)x100] was also reduced (77 % at 150 mg/kg bw/day versus 90.5 % in controls), however there was no clear evidence to indicate that this was due to treatment with chlorophene as no cross-fostering was carried out. Several deficiencies were reported for this study, including a lack of individual animal data making it difficult to ascertain whether the effects observed were chlorophene-related or not.

Classification for effects on lactation is warranted when clear evidence of an adverse effect in offspring due to transfer in milk or effects on milk quality are observed. In the 1973 perinatal and lactation study presented in the dossier there was no such evidence. Therefore, RAC agrees with the DS that there should be **no classification** for effects on lactation.

4.10 Neurotoxicity

Not applicable

Chlorophene bears no structural similarity to organophosphates, carbamates or other known inducers of delayed neurotoxicity. Acute and repeated-dose studies in several species did not indicate the occurrence of neurotoxic effects and the rapid excretion of chlorophene precludes an accumulation of the compound.

4.11 Specific investigations: other studies

Supplementary studies on the active substance

A study on the induction of drug-metabolising enzymes by chlorophene was published by Kao *et al.* (1986). In this study, male F344 rats were treated orally with 500 mg/kg bw/day chlorophene (purity >99%) in corn oil on three consecutive days. Phenobarbital (PB), 3-methycholanthrene (3-MC) and pregnelone-16α-carbonitrile (PCN) were used as model substances. Livers and kidneys were harvested 24 h after the last treatment. Arylhydrocarbon hydroxylase (AHH), glutathione S-transferase (GST), N-demethylase, O-demethylase, and UDP-glucuronyl transferase (UGT) activities were determined. Cytochrome P450b (now known as CYP2B1) protein levels were determined by immunoblotting and radioimmunoassay. Other cytochrome P450 isozymes were separated and quantified using HPLC.

Treatment with chlorophene resulted in an increase in cytochrome P-450 content and an accompanying decrease in aryl hydrocarbon hydroxylase (AHH) activity in both liver and kidney microsomes. Several other drug-metabolizing enzymes were not affected by chlorophene treatment. However, in kidney, chlorophene induced NADPH-cytochrome-c reductase and UGT activities and caused a small increase in total cytochrome P-450 content and glutathione concentration.

The cytochrome P-450 isozymes induced by chlorophene were fractionated by HPLC. The HPLC profile following chlorophene treatment most closely resembled that seen after phenobarbital. Using an immunoblotting procedure and a radioimmunoassay, it was shown that the increase in cytochrome P-450 content in the liver after chlorophene treatment was, in part, due to an increase in the phenobarbital-inducible isozymes, CYP2B1 and 2B2.

In the kidney, the increase in total cytochrome P 450 content after chlorophene exposure was not due to an increase in CYP2B1 and 2B2. The decrease in AHH activity appeared to be caused by non-competitive inhibition of constitutive AHH activity by chlorophene. Chlorophene also inhibited benzphetamine demethylation, although to a lesser extent. The failure to observe an increase in benzphetamine demethylase activity *in vivo*, despite the induction of CYP2B1, was probably due to the concomitant induction and inhibition of drug-metabolizing enzymes by chlorophene.

4.12 Human information

Medical surveillance of manufacturing plant personnel involved in chlorophene production revealed no health complaints associated with potential exposure to chlorophene (confidential, 2007 / A6_12_1).

A single report of contact dermatitis from chlorophene exposure is reported in the literature (Sonnex and Rycroft, 1986). A 49-year old bar manager developed contact dermatitis against chlorophene from a glass cleaning product.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 32: Summary of relevant information on degradation

Method	Results	Remarks	Reference Doc III
OECD 301B, ready biodegradability test (CO_2 evolution)	Over 60 % degradation was observed during the test duration (28 days), but not within 10 days.	The two tests on ready biodegradability gave ambiguous results. The chlorophene concentration in the manometric respirometry test was high and inhibition might have taken place. The CO_2 evolution test is considered more relevant as the chlorophene concentrations were below the EC_{50} value. This study indicates that chlorophene is readily biodegradable, since the pass level of 60 % degradation was reached. However, the pass level was not reached within the 10 day window as required by OECD 301B/CLP.	A7.1.1.2.1_01 (Bealing and Watson, 2002)
OECD 301 F, ready biodegradability test (manometric respirometry)	Chlorophene is considered not readily biodegradable under the conditions of this test.		A7.1.1.2.1_02 (Reis, 2007a)
OECD 302B, inherent biodegradability (Zahn/Wellens test)	Inherently biodegradable	Despite the issues of adsorption (or dissipation) at the beginning of the test, the remaining DOC is eliminated to a large degree (92 %) and the overall conclusion "inherently biodegradable" is considered acceptable.	A7.1.1.2.2 (Reis, 2007c)
Test procedure taken from Loehr and Matthews, 1992	DT_{50} (dissipation): 21.4 d. A normalisation of the DT_{50} (23 °C) to 12 °C results in a DT_{50} (12 °C) of 51.6 d.	The study examines the primary biodegradation of chlorophene in a sandy silt loam soil under aerobic conditions and a temperature of 22-23 °C. There is no information about degradation products, bound residues, mineralisation and degradation pathways in the study.	A7.2.1 (Nitsche, 2011)
OECD proposal for a new test guideline 311, anaerobic biodegradation of chlorophene in anaerobically digesting sewage sludge.	No net carbon-production (as methane and carbon dioxide) was found. Chlorophene is considered not anaerobically biodegradable under the conditions of the test.	A study on the anaerobic degradation of chlorophene in pork liquid manure was also submitted as supporting information (Gerhardz, 2011). The study supports the result of the OECD 311 test.	A7.1.2.1.2 (Reis, 2007b)

5.1.1 Stability

5.1.1.1 Abiotic degradation

Hydrolysis

The hydrolysis of chlorophene in sterile aqueous buffered solutions at pH 4, 7 and 9 was studied according to test method EC C.7, degradation – abiotic degradation hydrolysis as a function of pH, Annex V, 92/69/EEC (Greenwood, 2003b). The concentrations of chlorophene were measured via HPLC-UV.

The preliminary test run at 50 °C indicated less than 10% hydrolysis after 5 days, therefore in accordance with the guideline no further testing is required, and chlorophene is considered hydrolytically stable at the tested pH values. The highest loss was observed at pH 7 (6.5% in 5 days). The correlation coefficients of the hydrolysis slopes at pH 4, 7 and 9 (concentration as a function of time) range from 0.0369 to 0.1159, therefore any further extrapolation is difficult. The estimated half-lives of chlorophene at pH 7 and 9 were 44.4 and 37.4 days, but as they are based on the slopes with weak correlation coefficients, they must only be viewed as indicative.

Results of the test are summarised in the following table.

Please see Document III-A7.1.1.1.1 for further details on this study.

Table 33: Hydrolysis of chlorophene

Guideline / Test method	pH	Temp. [°C]	Initial TS concentration, C_0 [mg/L]	Reaction rate constant, K_h [d]	Half-life, DT_{50} [d]	Coefficient of correlation, r^2	Reference Doc III
EU method Annex V, C7 (92/69/EEC)	4, 7, 9	50	62.5 mg a.s./L	pH 4: no dissipation pH 7: 0.0156 day^{-1} pH 9: 0.0186 day^{-1}	pH 4: no dissipation pH 7: 44.4 days pH 9: 37.4 days	pH 4: 0.0369 pH 7: 0.0862 pH 9: 0.1159	A7.1.1.1 (Greenwood, 2003b)

Photolysis in water

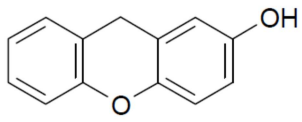
A study on photolysis of chlorophene in water was conducted according to the OECD proposal for a test guideline on phototransformation of chemicals in water – direct and indirect photolysis, draft August 2000 (Meinerling and Herrmann, 2007). The structure of the major photolysis product was elucidated in a follow-up study, by the combination of NMR, MS and UV/VIS spectroscopy (Freudenberger and Wesener, 2011), and the maximum concentration of this major photolysis product was estimated on the basis of the response of the test item in the UV chromatogram (Meinerling, 2011).

Chlorophene was incubated in aqueous buffer solution (pH 7) at a concentration of 5 mg/L over a time interval of 5 days (first experiment) and 4 hours (second experiment). The temperature during the study was maintained within the range of 20 to 30 °C. For technical reasons, the temperature during the first experiment was > 30 °C. Samples were either kept in the dark or exposed to a filtered xenon arc lamp. Residues of chlorophene were analysed by HPLC.

The chlorophene concentration rapidly dropped from an initial value of 99.4 % to 0 % of applied dose after 48 hours in the 5 days experiment and from an initial value of 99.7 % to a low value of 4.3 % of applied dose at the end of the 4 hours experiment. The aqueous photolysis half-life of chlorophene was calculated to be 0.7 hours.

The major photolysis product of chlorophene was identified as 2-hydroxy-xanthene (9H-xanthen-2-ol), and its maximum concentration was estimated to be 2.7 mg/L after 3 hours of irradiation. This corresponds to a relative concentration of 52.9 % of the parent substance. Furthermore, investigation by LC-MS/MS showed that two components with masses 184 and 212 were present in an additional sample containing a higher concentration of chlorophene (50 mg/L). However, as this sample was irradiated for analytical investigations only and since the high concentration of chlorophene is of no environmental relevance, a further in-depth investigation of these metabolites was not carried out.

Figure 2: The major photolysis product of chlorophene, 2-hydroxy-xanthene (9H-xanthen-2-ol)



Molecular Weight = 198.22
Molecular Formula = $C_{13}H_{10}O_2$

Under non-irradiated conditions, the mean parent concentration is not significantly reduced. Results are summarised in the following table.

Please see Document III-A7.1.1.1.2 for further details in this study.

Table 34: Photodecomposition of chlorophene in water

Guide-line / Test method	Initial TS concen-tration	pH value of the medium	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE})	Reaction quantum yield (ϕ^c_E)	Half-life (DT_{50})	Degradation products	Reference Doc III
OECD, 2000	5 mg a.s./L	7	0.98 [1/h]	11.97 [1/day]	Not available	0.7 h (experi-mental)	2-hydroxy-xanthene (9H-xanthen-2-ol), max. conc.: 52.9% of parent	A7.1.1.1.2 (Meinerling and Herrmann, 2007 Meinerling, 2011 Freudenberger and Wesener, 2011)

Photolysis in air and vaporisation behaviour

The half-life of chlorophene in air due to indirect photodegradation, i.e. oxidation with photochemically produced hydroxyl radicals, was calculated using the software programme AOPWIN, v. 1.91, 2000 by U.S.-EPA (Fàbregas, 2006). AOPWIN is a QSAR model which

requires only a chemical structure to make these predictions. The half-life of chlorophene in the troposphere was calculated to be 21.7 hours with a degradation rate of $18E^{-12}$ $cm^3 \times molecule^{-1} \times s^{-1}$. These estimations were carried out with respect to the OH radical reaction only, using a 24-hour-day with 5×10^5 OH radicals/cm^3. According to these results (DT_{50} < 2 days), Chlorophene is rapidly degraded by photochemical processes and no accumulation of chlorophene in the air is to be expected.

The vaporisation of chlorophene from an inert surface (glass) was studied according to an internal test method (Nitsche, 2011). The test item was dissolved in isopropanol at a concentration of 1.043 mg/L, and 6.2 mL of this solution was put in petri dishes (three replicates). In addition, two petri dishes were filled with pure isopropanol and two were empty. All the dishes were stored on a desk surface in the working laboratory for 125 days, and were weighed at certain time intervals (15 times in total) throughout the test period. Temperature and humidity were also recorded.

After 125 days, the chlorophene quantity left on the glass surface was approximately 40 % of the amount originally applied. The vaporisation rate was linear (0.020 g chlorophene / $m^2 \cdot$ day). This study thus clearly indicates that chlorophene evaporates from an inert surface, though slowly.

Please see Document III-A7.3.1 and III-A7.3.2 for further details on this study.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data.

5.1.2.2 Screening tests

Ready biodegradability

Chlorophene was investigated for its ready biodegradability in two different tests, a CO_2 evolution test according to OECD test guideline 301 B (Bealing and Watson, 2002) and a manometric respirometry test according to OECD test guideline 301 F (Reis, 2007a).

The CO_2 evolution test was performed with an initial test substance concentration of 10 mg a.s./L and activated sludge with a suspended solids concentration of 30 mg/L. The pass level of 60 % $ThCO_2$ was reached within the test duration of 28 days. However, CO_2 production failed to satisfy the 10 day window requirement. After a lag phase of less than 4 days, there was a rapid degradation of chlorophene corresponding to a ThCO2 evolution of just below 60 % (mean). Further degradation was inhibited for around 14 days, where after the $ThCO_2$ evolution reached 68 % (mean) of the theoretical maximum at the applied concentration over the course of the 28 day incubation. A similar behaviour was observed in the toxicity control, where the rate of CO_2 evolution initially kept pace with that observed in the reference control, but declined and stopped during a plateau phase between days 14 and 18 before restarting and continuing gradually until termination of the study. The inhibition occurred only after chlorophene was extensively degraded and may be caused by an unidentified degradation product of chlorophene. Alternatively, this could be explained by a second lag-phase required for the adaptation to degradation of a metabolite. In conclusion, over 60 % degradation of chlorophene was observed, but the rate of degradation did not

meet the criterion of 60 % degradation within 10 days from the onset of biodegradation (i.e. when 10 % degradation is reached) as required by OECD 301B/CLP.

Please see Document III-A7.1.1.2.1_01 for further details on the CO_2 evolution study.

In the manometric respirometry test the ready biodegradation of chlorophene was tested at an initial test substance concentration of 102 mg a.s./L and activated sludge with a suspended solids concentration of 30 mg/L. The degradation rate of chlorophene based on BOD was found to be 4 % (mean) after 14 days of incubation and 9 % (mean) after 28 days, at the end of the test. The degradation rate of chlorophene did thus not reach the pass level for ready biodegradability of 60 % based on BOD, and chlorophene is considered not readily biodegradable under the conditions of this test.

The level of test substance in the solution at the end of the test was high (81 % of the initial concentration), therefore adsorption to the activated sludge was not supposed to be of major influence. The possible degradation productions 4-chlorophenol and 4-chlorocatechol were not found on day 14 sampling, but on day 28, minor amounts of 4-chlorophenol (about 20 µg/L) were identified. In the toxicity control, the degradation of chlorophene was similarly low, but degradation of the reference substance was not inhibited. The test substance concentration was above the EC_{50} value for microorganisms (59.6 mg/L), therefore it is likely that the chlorophene inhibited the microorganisms in the activated sludge.

Please see Document III-A7.1.1.2.1_02 for details on the manometric respirometry study.

In conclusion, the two tests on ready biodegradability gave ambiguous results. The chlorophene concentration in the manometric respirometry test was high and inhibition might have taken place. Therefore, also assuming lower concentrations of chlorophene in STPs, the results of the CO_2 evolution test are considered more realistic. Here, 60 % degradation of chlorophene was observed during the 28 day test, but the level of 60 % degradation was not reached within the 10 day window.

Inherent biodegradability

The inherent biodegradability of chlorophene was tested in a Zahn/Wellens test according to OECD test guideline 302B (Reis, 2007c). The initial concentration of chlorophene was 87.4 mg a.s./L and the concentration of the activated sludge suspended solids was 200 mg/L. Based on DOC measurement, and after an initial rapid decline, the chlorophene level after 28 days was 7.8 % of the 3-hour concentration. This corresponds to a 92 % biodegradation. Chlorophene was thus found to be inherently biodegradable under the conditions of this test.

The DOC of the test flasks at 0 hours was 37-49 % of the initial concentration. Such a decline of chlorophene in the solution was also observed in the toxicity control. The recovery of chlorophene in the abiotic control without inocolum, however, was 100 %. This indicates that chlorophene adsorbed to the inoculum. Extraction of the activated sludge with acetonitrile resulted in very low recoveries; therefore it is likely that there is a strong, non-extractable binding of chlorophene (or primary degradation products) to the inoculum.

Despite these issues of adsorption (or dissipation) at the beginning of the test, the remaining DOC is eliminated to a large degree (92 %) and the overall conclusion "inherently biodegradable" is considered acceptable. The test item concentration was above the EC_{50} for microorganisms, but the

high level of inoculum might have facilitated degradation, even if the inoculum might have been inhibited to some degree.

Please see Document III-A7.1.1.2.2 for further details on the inherent biodegradation study.

The results of the ready and inherent biodegradation studies are summarized in the table below.

Table 35: Ready and inherent biodegradation of chlorophene in laboratory tests

Guide-line/ Test method	Test type[1]	Test para-meter	Inoculum			Add. subs-trate	Test substance conc.	Degradation		Reference Doc III
			Type	Concen-tration	Adap-tation			Incu-bation period	Degree [%]	
OECD 301B	Ready	CO_2	domestic sewage	0.03 g/L susp. solids	no	no	10 mg/L (nominal)	28 d	66-69%	A7.1.1.2.1_01 (Bealing and Watson, 2002)
OECD 301F	Ready	DOC or BOD	domestic sewage	0.03 g/L susp. solids	no	no	102 mg/L	28 d	9%	A7.1.1.2.1_02 (Reis, 2007a)
OECD 302B	In-herent	DOC and HPLC	industrial sewage	0.2 g/L susp. solids	Yes	no	87.4 mg/L	28 d	≥ 97%	A7.1.1.2.2 (Reis, 2007c)

[1] Test on *inherent* or *ready* biodegradability according to OECD criteria

An article from open literature (Swischer and Gledhill, 1973; Doc III A7.1.1.2.1_03, non-key study) is also available as supplementary information on the biodegradation of chlorophene. In this publication, the biodegradation of chlorophene (initial concentrations ranging from 0.1 to 20 mg/L) was studied in river water and three types of sludge systems over four weeks. A CO_2 evolution to the extent of approximately 60 % within the four week test period was observed. The study has shortcomings as several parameters were not reported. Nevertheless, it provides supporting information that biodegradation is expected under natural conditions.

5.1.2.3 Simulation tests

Aerobic degradation

There are no surface water simulation test data available.

The primary aerobic biodegradation of chlorophene in a single soil was studied according to a test procedure taken from Loehr and Matthews, 1992 (Nitsche, 2011).

The primary biodegradation of chlorophene in a sandy silt loam soil under aerobic conditions and a temperature of 22-23 °C was tested. The nominal concentration of the test substance was 10 mg/kg soil (analytically determined to be 8.6 mg/kg wet soil). The concentration of chlorophene and a reference substance (4-chloro-3-methylphenol) in the soil was monitored for 68 days using a substance specific HPLC analytical method. At the end of the test period, the test substance was dissipated for more than 85 % and a dissipation half-life of 21.4 days was derived. Normalisation of the DT_{50} (23°C) to 12°C results in a DT_{50} (12 °C) of 51.6 days.

There is no information about degradation products, bound residues, mineralisation and degradation pathways in this study. However, it shows the primary degradation of chlorophene and is considered acceptable for deriving a dissipation DT_{50} (12 °C) in soil of 51.6 days for the compound.

The results are summarized in the table below.

Please see Document III-A7.2.1 for further details on this study.

Table 36: Aerobic biodegradation of chlorophene in soil

Guideline / Test method	Test parameter	Test duration	Soil type	Test conditions	Initial test substance conc.	Results	Reference Doc III
Internal method	Concentration (HPLC measurements)	68 days	Sandy silt loam Total carbon: 1.1 % Humus: 1.9 % Clay: 12.9 % Sand: 53.9 % pH: 6.9 Moisture content: 80 % of field capacity	Temp: 22-23°C Light conditions: Darkness	10 mg/kg wwt (nominal) 8.6 mg/kg wwt (measured)	DT_{50} = 21.4 d (primary degradation/ dissipation)	A7.2.1 (Nitsche, 2011)

Anaerobic biodegradation

The anaerobic biodegradation of chlorophene in anaerobically digesting sewage sludge was assessed according to the OECD proposal for a new test guideline 311 (Reis, 2007b).

A manometric test was conducted with a nominal chlorophene concentration of 140 mg/L over a period of 60 days at 30-37 °C in darkness. No net carbon-production (as methane and carbon dioxide) was found. The reference item sodium benzoate was found to be anaerobically biodegradable, but in the toxicity control containing both chlorophene and the reference substance, no biodegradation was observed. Chlorophene can thus be assumed to inhibit the digesting sludge inoculum. Chlorophene is considered not anaerobically biodegradable under the conditions of this test. The results are summarized in the table below. Please see Document III-A7.1.2.1.2 for further details on this study.

An anaerobic study with Preventol BP® (chlorophene) in pork liquid manure was also submitted as supporting information (Gerhardz, 2011; no study summary is provided, non-key study). Chlorophene was found to be degraded moderately during the first 20 days of the anaerobic test period, to a level of approximately 76 % of the initial concentration at day 20. Thereafter, the decrease of the chlorophene concentration was decelerated to an insignificant extent reaching a level of approximately 70 % of the initial concentration after 64 days. The study indicates that anaerobic biodegradation of chlorophene cannot be expected under conditions of the test system, and it thus supports the results of the study on anaerobically digesting sewage sludge.

Table 37: Anaerobic degradation of chlorohene in sewage sludge

Guideline /Test method	Test para-meter	Inoculum			Test conditions	Initial test substance conc.	Degradation		Reference Doc III
		Type	Concen-tration	Adap-tation			Incu-bation period	Degree [%]	
OECD 311 (proposal, anaerobic biodegra-dation	CH_4 and CO_2 evo-lution	Anaerobically digesting sludge from a domestic STP in Darmstadt, Germany	2.1 g/L susp. solids	no	Temp: 30-37°C pH: 7.3-7.4 Light conditions: Darkness	140 mg/L (nominal)	60 d	0 %	A7.1.2.1.2 (Reis, 2007b)

5.1.3 Summary and discussion of degradation

5.1.3.1 Conclusion on abiotic degradation

It is not expected that hydrolytic processes will contribute to the degradation of chlorophene in the aquatic systems. Hydrolysis is not a major degradation pathway for chlorophene.

The aqueous photolysis half-life of chlorophene was calculated to be 0.7 hours. Therefore, photolysis will significantly contribute to the overall degradation of chlorophene in aquatic systems. The major degradation product is 2-hydroxy-xanthene (9H-xanthen-2-ol).

The calculated half-life of chlorophene in the troposphere is < 2 days (21.664 hours), and therefore no accumulation in the air is to be expected. Based on the vapour pressure and the Henry's Law constant (calculated, 3.7×10^{-3} Pa m³/mol), no significant volatilisation of chlorophene from water is to be expected. However, the study on vaporisation of chlorophene from an inert surface shows that evaporation does occur, but with a slow rate.

5.1.3.2 Conclusion on biodegradation

The two tests on ready biodegradability (Bealing and Watson, 2002 and Reis, 2007a) show dissimilar results. One test showed not ready biodegradability. This test was performed with high concentrations that are not considered environmentally relevant and which are above the EC_{50} for microorganisms. The other test, which is considered more relevant, indicated that chlorophene is readily biodegradable, since the pass level of 60 % degradation was reached. However, the pass level was not reached within the 10 day window as required by OECD 301B/CLP. In this test, the chlorophene concentrations were below the EC_{50} value.

Also in the inherent biodegradability test (Reis, 2007c), the chlorophene concentration was above the EC_{50} for microorganisms. However, the inoculum concentration was very high, and this might have compensated for a partial inhibition. The results from this study indicate that > 50 % of the initially applied chlorophene was rapidly and strongly bound to the inoculum, and could not be extracted with acetonitrile. After the initial rapid decline, 92 % of the available DOC was biodegraded at the end of the test. Chlorophene is thus considered inherently biodegradable.

The study on aerobic biodegradation in soil (Nitsche, 2011) indicates a primary degradation/dissipation of more than 85 % of the initially applied chlorophene at the end of the test period of 68 days. A half-life in soil of 51.6 days (12 °C) was derived.

In the study on anaerobic biodegradation in sewage sludge (Reis, 2007b), no net carbon-production (as methane and carbon dioxide) was found. Chlorophene is therefore considered as not anaerobically biodegradable.

Based on the availabe data, the substance cannot be considered rapidly biodegradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The adsorption/desorption behaviour of chlorophene in soil was investigated in two tests; a screening test according to OECD test guideline 121 (Jungheim, 2006b) and a test using four different soil types according to OECD test guideline 106 (Meinerling, 2007b).

In the screening study, the adsorption coefficient K_{oc} of chlorophene on soil was estimated using High Performance Liquid Chromatography (HPLC). Six reference standards of known K_{oc} were analysed on a HPLC system to determine an average capacity factor k'. Sodium nitrate was used to determine the HPLC system dead time (t_0). A regression curve with determined k' values and the known K_{oc} values (log k' versus log K_{oc}) was plotted.

Chlorophene was analysed on the same HPLC system during the same sample sequence as the reference substances. The K_{oc} value for chlorophene was estimated by interpolation from the reference substance regression line. The estimated K_{oc} value for chlorophene is log 3.43 (K_{oc} = 2511), further data is given in the table below. According to OECD, the K_{oc} estimated according to OECD test guideline 106 has a higher reliability. However, the output from an OECD 121 test is acceptable as an estimation of a chemical's partitioning behaviour.

Please see Document III-A7.1.3 for further details on the screening study.

Table 38: HPLC retention times and Koc values for chlorophene and reference substances, OECD 121

Calibration substance	Retention time [tr] = min*	Capacity factor k'*	Log k'*	Log Koc	Reference Doc III
2-Nitrobenzamide	5.535	0.678	- 0.169	1.45	A7.1.3 (Jungheim, 2006b)
3-Nitrobenzamide	5.680	0.722	- 0.141	1.95	
3,5-Dinitrobenzamide	6.018	0.824	- 0.084	2.31	
Triazoxide	7.389	1.240	0.093	2.44	
Naphthalene	8.276	1.509	0.179	2.75	
Phenanthrene	9.550	1.896	0.278	4.09	
Chlorophene (Test item)	**8.532**	**1.587**	**0.201**	**3.43**	

* mean value from 3 single values

In the OECD 106 test on four soil types (Meinerling, 2007b), the soils comprised of a sand, a loamy sand, a clay loam and a loam, respectively (corresponding to the soils Lufa 2.1, 2.2, 6S and Eurosoil 3). Aqueous solutions of the test item were equilibrated with the four soil types and the adsorption and desorption coefficients and constants were determined. The concentration of the test item was determined using HPLC methods.

According to the guideline, the OECD 106 study consists of a preliminary study, screening tests for adsorption and desorption, and the determination of Freundlich adsorption isotherms.

From results of the preliminary tests, a soil/solution ratio of 1:25 (2 g soil and 50 mL aqueous 0.01 M $CaCl_2$ solution of the test item) and a 48 h incubation time (to reach adsorption equilibrium) were chosen.

In the adsorption and desorption screening tests, desorption was estimated at one concentration of the test item using 4 different soil types. At desorption equilibrium, desorption of the adsorbed test item from the different soil types ranged from 18% up to 45% desorption. Adsorption was measured and the distribution coefficients K_d were in the range of 19 to 115 mL/g.

The Freundlich adsorption coefficients (K_F^{ads} values) varied between 31.8 and 145 $\mu g^{1-1/n}(cm^3)^{1/n}g^{-1}$. This relates to average distribution coefficients between 19 (Lufa 2.1) and 115 mL/g (Eurosoil3) which correspond to the values determined in the screening test.

Freundlich coefficients determined for the desorption (K_F^{des} values) were higher than those determined for the adsorption (range of K_F^{des}: 66.0-190.3 $\mu g^{1-1/n}(cm^3)^{1/n}g^{-1}$).

New Distribution coefficients, K_d, may be derived for the lowest test concentration for soil and water using the Freundlich equation, $C_{soil} = K_d^F * C_{water}^{1/n}$ with K_d^F being the Freundlich distribution coefficient and 1/n the Freundlich exponent. Since c_{soil} is equal to $K_d * c_{water}$, the new Kd is $K_d^F * C_{water}^{1/n-1}$. From the new K_d, new K_{oc} values can be calculated by using the equation $K_{oc} = K_d/f_{oc}$ with f_{oc} being the fraction organic carbon. The mean value of the four soil types for K_{oc} is used for PEC calculations as the tested concentrations are closest to the estimated environmental concentrations.

Table 39: Koc values at the lowest tested soil concentrations, OECD 106

Soils	Test conc. in water	Testconc. in soil	Kd	Koc
Eurosoil3	0.73	111	156	4726
Lufa 6S	1.44	93	64	3490
Lufa 2.2	1.51	103	75	3165
Lufa 2.1	2.51	63	25	2210
Mean value for Koc				**3398**

In conclusion, the resulting K_{oc} value derived from this study is 3398.

Please see Document III-A7.2.3.1 for further details on this study.

5.2.2 Volatilisation

Atmosphere

No atmospheric effect study is available as it is not required for biocides used as disinfectant (product types 2 and 3). Furthermore, based on the Henry's Law constant (calculated, 3.7 x 10^{-3} Pa×m³/mol; Fàbregas, 2006), no significant volatilisation of chlorophene from water is to be expected. The vaporisation of chlorophene from an inert surface (glass) was studied according to an internal test method. Results show that evaporation of chlorophene occurs; however quite slowly. Calculations of the chemical lifetime in the troposphere resulted in a half-life of 21.7 hours. According to these results (DT_{50} < 2 days), chlorophene is rapidly degraded by photochemical processes and no accumulation of chlorophene in the air is to be expected.

Please see Document III-A7.3.1 for further details on the calculation.

5.2.3 Distribution modelling

No data.

5.3 Aquatic bioaccumulation

5.3.1 Aquatic bioaccumulation

Table 40: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference Doc III
OECD 305, bioconcentration factor in fish (*Danio rerio*)	107-110 (whole body) 1401-1130 (lipid normalised)	The lipid content of the fish increased by more than 25 % from the start to the end of the study. A higher lipid content gives a lower BCF value (lipid normalised), but on the other hand, a higher lipid content may lead to a higher concentration (wet weight) at steady state and thus a higher BCF based on whole body wet weight. In conclusion, the measured steady-state whole body wet weight BCF values of around 100 L/kg are considered valid. 24 h after initiation of the depuration phase, no chlorophene was detected in any of the fish samples.	A4.3.3.1 (Confidential, 2009)

5.3.1.1 Bioaccumulation estimation

The aquatic BCF of chlorophene was estimated using the QSAR-approach as recommended in the Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances; Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances; Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market (European Commission, 2003).

Based on a measured log Kow value of 4.275, the BCF-value of chlorophene was calculated to 858.5.

However, since e study on bioconcentration in fish is available (see below), this result is only included for information purposes.

Please see Document III-A7.4.2 (Fàbregas, 2007) for further details on the calculation.

5.3.1.2 Measured bioaccumulation data

The bioconcentration factor (BCF) of chlorophene in fish was determined in a study conducted according to OECD test guideline 305 (Confidential, 2009).

Groups of 37 and 47 individuals of *Danio rerio* were exposed to chlorophene for 8 days (uptake phase) at nominal concentrations of 3 and 15 µg/L, respectively. On day 9, the depuration phase was initiated, i.e. the fish were transferred to water with no chlorophene. The depuration phase lasted for 7 days. As pre-tests indicated that the bioconcentration of the test item might be fast and low, the study was carried out over only 15 days and with a reduced number of fish and water samples for analysis. The number of control fish taken for analysis was also reduced. Samples of the water and of fish were taken on days 1, 4, 6, 8, 9, 11 and 13, and the steady-state BCF was calculated as the ratio of the chlorophene concentrations in fish and water. In the fish samples during the uptake phase, the percentage of applied chlorophene in the treatment groups of 3 and 15 µg/L were 0.329-0.395 % and 1.433-2.046 %, respectively (HPLC determinations). 24 h after initiation of the depuration phase, no chlorophene was detected in any of the fish samples. For the treatment groups of 3 and 15 µg chlorophene/L, the steady-state BCF in terms of total bodyweight (wet weight) was 107 and 110 L/kg, respectively. In terms of lipid content, the steady-state BCF was 1401 and 1130 L/kg, respectively. Because of the fast uptake and elimination kinetics, no kinetic BCF could be determined.

The lipid content of the fish increased by more than 25 % from the start to the end of the study. A higher lipid content gives a lower BCF value (lipid normalised), but on the other hand, a higher lipid content may lead to a higher concentration (wet weight) at steady state and thus a higher BCF based on whole body wet weight. In conclusion, it is assumed that the increase in lipid content did not impair the test and that the measured steady-state whole body wet weight BCF values of around 100 L/kg are considered valid. The results are summarised in the table below. Please see Document III-A7.4.3.3.1 for further details on this study.

Table 41: Bioconcentration factors obtained for chlorophene

Test method	Test species	Exposure time	Log K_{ow}	Concentration of a.s. [µg/L]	BCF (L/kg)	Reference Doc III
Measured steady-state BCF according to OECD 305	*Danio rerio*	15 days	4.275	3 15	107 110 (whole body) 1401 1130 (lipid normalised)	A7.4.3.3.1 (Confidential, 2009)

5.3.2 Summary and discussion of aquatic bioaccumulation

The log K_{ow} value for chlorophene is 4.27. According to the TGD (2003), values greater than or equal to 3 indicate that the substance may bioaccumulate. However, the available fish bioconcentration study according to OECD 305 results in a measured steady-state BCF_{fish} based on whole body and lipid content of 110 L/kg and 1401 L/kg, respectively. However, no chlorophene could be detected in the fish samples 24 h after initiation of the depuration phase.

Based on this information, chlorophene is not expected to bioaccumulate in the environment.

5.4 Aquatic toxicity

Studies on the acute effects of chlorophene on fish and aquatic invertebrates are available. However, these are not considered valid due to insufficient reporting and the fact that only two test concentrations were used, from which no reliable LC_{50} or EC_{50} could be established. Please see sections 5.4.1.1 and 5.4.2.1 and Document III-A7.4.1.1 (non-key study) and III-A7.4.1.2 (non-key study) for further details.

However, chronic studies are available and the results of these tests are valid. The chronic toxicity towards fish was studied according to OECD 210. **This study resulted in a NOEC (mortality) of 0.58 µg a.i./L, the lowest NOEC among the chronic aquatic toxicity test indicators and the value used for the classification with H410: Very toxic to aquatic life with long lasting effects.**

The chronic toxicity towards daphnids was tested according to OECD 211 and a NOEC (reproduction) of 6.7 µg a.i./L was determined.

Growth inhibition of algae was tested according to OECD 201. The study resulted in an E_rC_{50} value of 197.2 µg a.i./L and a NOErC value of 103.6 µg ai./L. **The E_rC_{50} value of 197.2 µg a.i./L = 0.197 mg a.i./L gives a classification with H400: Very toxic to aquatic life.**

The inhibition of aquatic microbial activity was investigated according to OECD 209. In this study, an EC_{50} for aquatic microorganisms of 59.6 mg a.i./L was derived.

Table 42: Summary of relevant information on aquatic toxicity

Guideline / Test method	Results	Remarks	Reference Doc III
OECD 210, early life stage test with zebrafish (*Danio rerio*)	NOEC (mortality) = 0.58 µg/L = 0.00058 mg/L	Criteria for classification as H410, aquatic chronic 1, fulfilled by $NOEC_{fish}$ and $NOEC_{daphnia}$. Criteria for classification as H400, aquatic acute 1, fulfilled by $E_rC_{50\ algae}$.	A7.4.3.2 (Confidential, 2008)
OECD 211, *Daphnia magna* reproduction test	NOEC (reproduction) = 6.7 µg/L = 0.0067 mg/L		A7.4.3.4 (Weyers, 2007)
OECD 201, growth inhibition of *Pseudokirchneriella subcapitata*	E_rC_{50} = 197.2 mg/L = 0.197 mg/L NOEC (growth) = 0.104 mg/L		A7.4.1.3 (Egeler et al., 2006)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

A study on the acute toxicity of chlorophene towards fish (*Danio rerio*) in a static water system was submitted, but was not considered valid. The study was insufficiently reported, i.e. there was a lack of reporting of several test system details and test conditions (e.g. dissolved oxygen in the water and replicates). Furthermore, only two chlorophene concentrations were tested, 1.3 mg/L and 1.8 mg/L. No effects occured in the lower treatment group, but at 1.8 mg/L, 100 % of the fish died. This considerable difference in effects at such close concentrations seems questionable. The derivation of a reliable LC_{50} value would require a better and more thoroughly reported test system, and also more than two test concentrations. No statistical analyses could be carried out with only two test concentrations. There were also no measurements of the actual chlorophene concentrations. Please see Document III-A7.4.1.1 (non-key study) for further details.

However, a valid chronic toxicity study was submitted and the results from this tests is used for the purpose of the CLH proposal. Therefore, no new acute toxicity studies were requested.

5.4.1.2 Long-term toxicity to fish

The long-term toxicity of chlorophene has been tested in an early-life stage test with the Zebra-fish (*Danio rerio*) according to OECD test guideline 210 (Confidential, 2008).

Fertilised eggs were exposed under flow-through conditions to nominal concentrations of 0 (control), 0.95, 3.05, 9.77, 31.3 and 100 µg chlorophene/L. The test duration was 30 days after hatching of the larvae. The chlorophene concentrations were analytically determined prior to the initiation of the study, and at days 0, 6, 13, 20, 27, 34 and 37 for all test concentrations and the control. Mean measured concentrations ranged from 54 % to 81 % of the nominal concentrations, and therefore, analytically determined mean concentrations were used for the calculation and reporting of results. The daily recorded effects were mortality, hatching success, growth and symptoms of intoxication. The effect concentrations were statistically determined. The resulting lowest NOEC, based on mortality, was 0.58 µg test item/L (mean measured concentration). The LOEC was determined to be 1.65 µg test item/L (mean measured concentration).

Please see Document III-A.7.4.3.2 for further details on this study.

Table 43: Long-term toxicity of chlorophene to fish

Guideline/ Test method	Species	Endpoint/ Type of test	Exposure		Results, mean measured conc. [µg a.i./L]		Reference Doc III
			Design	Duration	NOEC	LOEC	
OECD 210 (1992)	*Danio rerio* (Zebra-fish)	Mortality, hatching success, symptoms of intoxication, growth parameters body weight and length of surviving fish	Flow-through	Until 30 days after hatching of larvae	Mortality: 0.58 Hatching: 73.0 Growth: 22.9 **Criteria for classification as H410, aquatic chronic 1 fulfilled by NOEC (mortality).**	1.65	A.7.4.3.2 (Confidential, 2008)

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

A study on the acute toxicity of chlorophene towards invertebrates (*Daphnia magna*) in a static water system was submitted, but as the acute fish study, it was not considered valid. This was mainly due to insufficient reporting (no information on holding and dilution water given, also no reported information on test conditions, e.g. pH, aeration of water, dissolved oxygen and temperature, and no information given on replicates) and the fact that only two concentrations were tested, 1.0 and 2.0 mg/L. The immobilisation in the two treatment groups were 0 % and 100 %, respectively, and these are not considered reliable results. The derivation of a reliable EC_{50} value would require a better and adequately reported test system, as well as more than two test concentrations. No statistical analyses could be carried out with only two test concentrations. There were also no measurements of the actual chlorophene concentrations. Please see Document III-A7.4.1.2 (non-key study) for further details.

However, a valid chronic toxicity study was submitted and the results from this tests is used for the purpose of the CLH proposal. Therefore, no new acute toxicity studies were requested.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A *Daphnia magna* reproduction study was conducted according to EEC Methods for Determination of Ecotoxicity Annex to Directive 97/548/EEC Part C, Method 20 '*Daphnia magna* Reproduction Test', corresponding to OECD test guideline 211 (Weyers, 2007).

The influence of chlorophene on the reproduction and survival rate of daphnids was investigated during 21 days in a semi-static system, with nominal chlorophene concentrations of 0.0032, 0.01, 0.032, 0.1 and 0.32 mg/L. The chlorophene concentrations were measured analytically on day 0, 7 and 14, and were in the range of 60-101 % and 30-95 % of the nominal values in the freshly

prepared and aged test, respectively. The results were therefore based on mean measured concentrations. The test parameters were survival of parent animals and number of offspring per adult.

A dose response relationship was observed for the number of offspring per adult, with no effects in the two lowest treatments. The NOEC and LOEC with regards to reproduction were determined to be 6.7 µg a.i./L 25.4 µg a.i./L. The NOEC and LOEC for mortality were ≥ 29.6 µg a.i./L.

Please see Document III-A7.4.3.4 for further details on this study.

Table 44: Reproduction test of chlorophene with *Daphnia magna*

Guideline / Test method	Endpoint/ Type of test	Exposure Design	Exposure Duration	Results, mean measured conc., [µg a.i./L] NOEC/ LOEC		Reference Doc III
EEC Method 20 (2001) (equal to OECD 211, 1998)	reproduction	Semi-static	21 d	6.7/ 25.4	reproduction	A7.4.3.4 (Weyers, 2007)
				≥29.6/ >29.6	mortality	
					Criteria for classification as H410, aquatic chronic 1 fulfilled by NOEC (reproduction)	

5.4.3 Algae and aquatic plants

The influence of chlorophene on the growth of the green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was investigated in a 72 hours static test according to OECD test guideline 201 (Egeler et al. 2006).

P. subcapitata were exposed to nominal concentrations of 0.0048, 0.0153, 0.0488, 0.1563 and 0.5000 mg chlorophene/L. The chlorophene concentrations were measured analytically at 0 and 72 hours. The mean measured concentrations ranged from 82 % to 107 % of the nominal test concentrations at the beginning of the test and from 35 % to 39 % at the end. Therefore, all the results were based on mean measured concentrations. The calculated average growth rates decreased in a dose dependent manner. Effect concentrations were determined statistically. A clear dose-response relationship could be derived from the algae concentration data. The 72-hour E_rC_{50} value (growth rate inhibition) was 197.2 µg a.i./L and the 72 h NOE_rC value was 103.6 µg a.i./L. A deviation from the OECD 201 guideline is that the nutrient concentrations in the test medium were higher than recommended. However, there are no reasons to expect interactions between the test material and constituents of the test medium, and the test is therefore still regarded as reliable.

Please see Document III-A7.4.1.3_01 for further details on this study.

A second algae growth inhibition study is also available, which was not considered valid due to major deviations from the guideline and poor documentation of the study. A guideline which reportedly resembles the OECD 201 guideline was used. However, there were several deviations from the latter guideline. The information on test conditions and culture medium was very limited

or lacking, and not sufficient to assess the validity criteria of OECD 201. There were not enough test concentrations, and no monitoring of the chlorophene concentrations during the test. No information on replicates is given. The studied test parameter was cell number (algal biomass), and the effect concentration, E_bC_{50}, was determined graphically to be 0.038 mg/L, based on nominal concentrations. The initial cell concentration was not determined, the cell numbers were only counted at the end of the test (72 hours). No statistical analysis was carried out. Due to these deficiencies, only the first alga growth inhibition study (Egeler et al., 2006) is considered for CLH purposes and is presented in the table below.

Please see Document III-A7.4.1.3_02 for details.

Table 45: Toxicity of chlorophene to algae

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results, mean measured conc. [mg a.i./L]**			**Reference Doc III**
			Design	**Duration**	**NOEC**	**E_bC_{50}[1]**	**E_rC_{50}[2]**	
OECD 201 (2002)	*Pseudokirch-neriella subcapitata*	Growth inhibition	Static	72 h	0.0323 (biomass) **0.1036** (growth) **Criteria for classification as H400, aquatic acute 1 fulfilled by E_rC_{50}.**	0.1314	**0.1972**	A7.4.1.3 (Egeler et al., 2006)

[1] calculated from the area under the growth curve; [2] calculated from growth rate

5.4.4 Other aquatic organisms (including sediment)

Inhibition of microbial activity (aquatic)

The effect of chlorophene on aquatic micro-organisms was investigated according to ISO Guideline 8192 (1986), which equals OECD test guideline 209 (Caspers and Müller, 1991).

Activated sludge was exposed to chlorophene concentrations of 10, 18, 32, 56 and 100 mg/L. The endpoint investigated was inhibition of the respiratory rate. Chlorophene showed inhibitory effects on the microbiological activity of activated sludge, and the EC_{50} was determined to be 59.6 mg a.i./L.

The results are based on nominal chlorophene concentrations, as there were no concentration measurements during the study. The EC_{50} value was not confirmed by statistical analysis and the results of the controls and reference substance are not reported. The test is still considered valid as inhibitory effects on microorganisms have been seen in biodegradation studies at concentrations in the same order of magnitude as the EC_{50} from this study.

Please see Document III-A7.4.1.4 for further details on this study.

Table 46: Influence of chlorophene on inhibition of microbial activity (aquatic)

Guideline/ Test method	Species/ Inoculum	Endpoint/ Type of test	Exposure		Results [mg a.i./L]			Reference Doc III
			Design	Duration	EC_0	EC_{10}	EC_{50}	
ISO 8192 (1986) (equal to OECD 209)	Activated sludge	Oxygen consump-tion	Respira-tion inhibition	3 hours	< 8.22	20	59.6	III-A7.4.1.4 (Caspers and Mueller 1991)

Effects on sediment dwelling organisms

Testing on sediment-dwelling organisms is not a product-type specific requirement for PTs 2 and 3. The log Kow for chlorophene is above the trigger value for a sediment assessment, and indirect emissions to water bodies can occur via effluent water from STPs and due to run-off from soil. An assessment using the equilibrium partitioning method was considered sufficient.

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

The criteria are taken from the Guidance on the Application of the CLP Criteria (ECHA, 2012).

- Rapid biodegradation:
    - The result from the manometric respiration test was: BOD 4 % (mean) after 14 days of incubation and 9 % (mean) after 28 days. This means that chlorophene does not meet the criteria. However, the concentration of chlorophene was above the EC_{50} value for microorganisms (59.6 mg/L), therefore it is likely that the substance inhibited the microorganisms in the activated sludge.
    - The result from the CO_2 evolution test was: CO_2 reached 68 % of the theoretical maximum at the applied concentration over the course of the 28 day incubation. CO_2 production failed to reach the pass level within the ten-day window. Chlorophene did not degrade biotically in the aquatic environment by > 70 % in 28 days, and the substance can therefore **not** be classified as rapidly biodegradable.
    - Regarding abiotic degradation, hydrolysis is not an important degradation way for chlorophene. Photolysis can be an important way of primary degradation. However the test is not an ultimate biodegradation test and cannot be used for classification purposes.
    - Simulation tests: There are no surface water simulation test data available. The soil simulation test shows primary degradation, but not ultimate degradation.

The main conclusion is that the substance **cannot** be classified as rapidly biodegradable.

- Potential of bioaccumulation:
    - log Kow > 4: Chlorophene meets the criterion.
    - BCF_{fish} based on whole body (110 L/kg) < 500: Chlorophene does not meet the criterion

Chlorophene has a high lipophilicity and has therefore a potential to bioconcentrate. However, experimentally derived BCF_{fish} (whole body) is preferred for classification purposes as such data overrides surrogate data such as a Kow value alone. In addition, in the fish BCF test no chlorophene could be detected in the fish samples 24 h after initiation of the depuration phase. Based on this, chlorophene does not meet the criterion for bioaccumulation.

- Aquatic toxicity:
    - Acute toxicity: The studies on acute toxicity towards fish and daphnids were not considered valid (please see sections 5.4.1.1 and 5.4.2.1 for more information), but there is a valid acute endpoint available for algae. The E_rC_{50} from the growth inhibition study on algae is < 1 mg/L (0.1972 mg/L) and gives the acute classification Category Acute 1.
    - Chronic toxicity: There are adequate chronic toxicity data available for all three trophic levels. The lowest chronic NOEC from the early life stage fish test is < 0.1 mg/L (0.00058 mg/l) and gives the chronic classification Category Chronic 1.

According to Annex 1: table 4.1.0 in the Guidance on the Application of the CLP Criteria, chlorophene meets the criterion for: (b) **Long-term aquatic hazard**. (i) **Non-rapidly degradable substances for which there are adequate chronic toxicity data available. Category Acute 1. Category Chronic 1.**

Substances with chronic toxicities below 0.1 mg/l (if non-rapidly degradable) contributes as components of a mixture to the toxicity of the mixture even at a low concentration and shall normally be given increased weight in applying the summation of classification approach. Therefore a multiplying factor (M-factor) has to be assigned. The M-factor can be taken from table 4.1.3 In Annex 1 to the Guidance on the Application of the CLP Criteria. The chronic M-factor for chlorophene is found in the table to be 100.

Regarding the acute toxicity classification, the acute M-factor is 1, according to table 4.1.3 in Annex I to the Guidance on the Application of the CLP criteria.

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

Symbol: GHS09

Signal word: WARNING

Aquatic Acute 1

H400: Very toxic to aquatic life

M-factor: 1

Aquatic Chronic 1

H410: Very toxic to aquatic life with long lasting effects

M-factor: 100

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Chlorophene is not included in Annex VI of the CLP regulation. The DS proposed to classify chlorophene as Aquatic Acute 1 with an M-factor 1 and Aquatic Chronic 1 with an M-factor 100.

Degradation

A hydrolysis study conducted according to the test method EC C.7 (Hydrolysis as a function of pH) showed that chlorophene is not hydrolysable at various pHs. An aqueous photolysis study was performed according to the OECD proposal for a test guideline on phototransformation of chemicals in water (2000) and the study showed that the chlorophene half-life is 0.7 hours. The major photolysis product of chlorophenewas identified as 2-hydroxy-xanthene (9H-xanthen-2-ol), and its maximum relative concentration was 52.9 % of the parent substance. The DS stated that photolysis will significantly contribute to the overall degradation of chlorophene in aquatic systems.

Three biodegradability screening tests were provided in the CLH report. Chlorophene was shown to biodegrade (60–68%) in a test conducted according to the OECD TG 301B but did not fulfil the requirement of a 10 day window. In the manometric respirometry test (OECD TG 301F) 9% of chlorophene was degraded within 28 days (<60%), thus it was considered not readily degradable. However, chlorophene was found to be inherently biodegradable under the conditions of OECD TG 302B.

An aerobic simulation test (a non-standardised test procedure taken from Loehr and Matthews, 1992) at various temperatures showed that the substance degrades slowly in aerobic soil having a DT_{50} = 21.4 days at 23°C and 51.6 days at 12°C. An anaerobic simulation test on sewage sludge (OECD proposal for a new TG 311) showed that no biodegradation takes place under anaerobic conditions.

Based on the provided degradation studies the DS concluded that chlorophene cannot be considered as a rapidly biodegradable substance.

Bioaccumulation

The reported log K_{ow} value for chlorophene is 4.27. The measured bioconcentration factor in fish (OECD TG 305, *Danio rerio*) was 107–110 L/kg. The lipid normalized value in the CLH dossier was given incorrectly in the CLH report (1401 and 1130 L/kg) and the DS clarified after public consultation that this value is incorrect, – it is not related to the whole body of the fish, but the fat tissue alone – and the valid lipid normalised BCF value after recalculation was 55–56 L/kg. Based on this value (and the fact that chlorophene is eliminated within 24 hours from the fish body), the DS concluded that chlorophene is not expected to bioaccumulate in the aquatic environment.

Aquatic toxicity

Acute toxicity studies for all three trophic levels were provided, however, the fish and daphnid tests were not considered to be valid by the DS. The proposed Aquatic Acute 1 classification was based on the growth inhibition of algae (OECD TG 201, *Pseudokirchneriella subcapitata*). Based on E_rC_{50} = 0.1972 mg/L an M-factor of 1 was proposed by the DS.

Chronic toxicity studies were reported for all the three trophic levels. A chronic toxicity study in zebrafish (*Danio rerio*), conducted according to OECD TG 210, resulted in a NOEC value of 0.00058 mg/L for mortality. A chronic study on *Daphnia magna* (OECD TG 211) resulted a NOEC value of 0.0067 mg/L for reproduction. The algae study conducted conducted according to OECD TG 201 (*P. subcapitata*) resulted in a NOEC (growth) of 0.1036 mg/L. The DS concluded that the chronic classification should be based on the fish toxicity, resulting in Aquatic Chronic 1 with an M-factor of 100.

Comments received during public consultation

The environmental part of the chlorophene CLH dossier was commented on by 6 MSCAs and one manufacturer. Most commenting MSCAs supported the DS's proposal but two MSs proposed a higher chronic M-factor.

One MSCA questioned whether the M-factor of 1 is appropriate if only one acute endpoint is available, and this acute endpoint is not for the most sensitive species (i.e. fish) according to the chronic tests. The same MSCA recommended equal M-factors for both acute and chronic classifications (i.e. 100).

Another MSCA also argued for a higher acute M-factor based on the results of chronic studies, where fish was the most sensitive species with a very low NOEC (0.58 µg/L). Therefore, the MS suggested that the acute M-factor of 1 based on the acute toxicity of algae (ErC50 = 0.197 mg/L and NOECr=0.104 mg/L) is too low.

Several comments concerned the incorrect lipid normalised BCF, recognised by the DS as a mistake in the CLH report: the originally reported BCF value was in the lipid fraction of the fish, and the correct value should be normalised for the whole body lipid content of the fish. The correctly calculated lipid normalised BCF (55–56 L/kg) is smaller than the measured, not normalised BCF (107–110 L/kg), and does not influence the final concluson on aquatic hazard classification.

The DS clarified two comments that concerned the results of the ready biodegradability tests: both tests showed that the substance is not readily biodegradable under the prevailing test conditions. In the CO_2 evolution test (OECD TG 301B) the pass level was not reached in the 10-day window, while in the manometric respirometry test (OECD TG 301F) degradation was low (9% after 28 days).

The validity of the chronic fish test was questioned by a manufacturer and a chronic M-factor of 10 was recommended. The validity of the test, and the justification for M (chronic) = 100 was thoroughly argued by the DS in the RCOM.

Assessment and comparison with the classification criteria

Abiotic degradation
Chlorophene may be considered photodegradable in air (QSAR estimate of AOPWIN) and in water (an OECD proposed TG from 2000, identical with OECD 316 from 2008) but not hydrolysable by EC C.7 (Annex V, 92/69/EEC).

Biodagradability
The OECD TG 301B test results showed that the pass level of ready biodegradability was not reached in the 10-day window and in the OECD TG 301F manometric respirometry test the degradation rate was 9% after 28 days. Both of these results confirm that chlorophene is not readily biodegradable.

Degradability of chlorophene in summary: not rapidly degradable.

Bioaccumulation
Based on the lipid normalised BCF of 55–56 L/kg (smaller than the not normalised measured value of 107–110 L/kg), chlorophene is not expected to bioaccumulate in the environment, being under the treshold: 55–56 L/kg < 500 L/kg (OECD TG 305, *Danio rerio*).

Aquatic acute toxicity
The only valid acute study, the algae study on growth inhibition (OECD TG 201,

Pseudokirchneriella subcapitata) resulted in an ErC_{50} value of 0.197 mg/L. This value is below 1 mg/L and is supported by the QSAR estimates reported under section *Supplemental information – In depth analyses by RAC*, resulting in a classification of Aquatic Acute 1 (H400) with an M-factor of 1, as the ErC_{50} falls within the range 0.1 < 0.197 mg/L < 1 mg/L.

Aquatic chronic toxicity
The chronic fish mortality study (OECD TG 210, early life stage test with zebrafish *Danio rerio*) showed the lowest NOEC value of 0.00058 mg/L, meeting the criterion for classification (non-rapidly degradable substance, NOEC<0.1 mg/L) as Aquatic Chronic 1 (H410) with the M-factor of 100 (0.0001 < 0.00058 mg/L <0.001 mg/L).

In conclusion, RAC agrees with the DS's proposal to classify chlorophene as:

- **Aquatic Acute 1 (H400: Very toxic to aquatic life) with and M-factor of 1; and**
- **Aquatic Chronic 1 (H410: Very toxic to aquatic life with long lasting effects) with an M-factor of 100.**

Supplemental information - In depth analyses by RAC

The classification of chlorophene as Aquatic Acute 1, with an M-factor of 1, is based on the only valid acute study, the algae growth inhibition test. The M-factor of 1 was considered too low in some comments received during public consultation, and a higher M-factor was recommended due to an incomplete data-set. In spite of the fact that further acute ecotoxicity data were not needed for the purposes of the biocide legislation, the M-factor cannot be determined with high certainty, and it might change if valid acute fish and/or invertebrate data become available in the future.

The CLP Regulation allows predicted data to be taken into account in the absence of measured data. QSAR estimates could provide additional information on fish and daphnid acute toxicity (e.g. LC_{50}), and ensure the determination of a more certain M-factor. Therefore, the following QSAR values were calculated based on different estimation methods:

ECOSAR v1.11

ECOSAR placed the substance in the class "Phenols" and produced the following two predictions:

Fish acute toxicity: LC_{50} (96h)=**0.8** mg/L

Daphnia acute toxicity: LC_{50} (48h)=**0.7** mg/L

Both predictions are considered to be in the applicability domain of the models as the logKow is below 7, the water solubility exceed the effect level and the molecular weight is below 1000.

Topkat

Topkat is a module which is part of the software Accelrys/Discovery studio. It contains QSAR models for acute toxicity to fish and daphnia.

Fish acute toxicity: LC_{50} (96h)=**1.5** mg/L

Daphnia acute toxicity: LC_{50} (48h)=**2.1** mg/L

Both predictions are in the Optimal Prediction Space meaning that they are considered as being in the applicability domain of the QSAR models.

EU-TGD (2003) based on the QSAR equation of Verhaar *et al*. (1995)

Fish acute toxicity: LC_{50} (96h)=**1.15** mg/L Daphnia acute toxicity: LC_{50} (48h)=**1.43** mg/L The applied model – created for chemicals acting by polar narcosis – is valid in the $logK_{ow}$ range of 1–6, for chemicals with a molecular weight less than 600 containing no iodine or ionic groups. Classes of chemicals which act by polar narcosis include phenols. A detailed definition of the domain has been described by Verhaar *et al*. (1995). The results of all three QSAR methods can be used as supportive information since the substance falls within the applicability domains of the models.

6 OTHER INFORMATION

7 REFERENCES

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
--	Eastin W. C. *et al.*	1998	The National Toxicology Program evaluation of genetically altered mice as predictive models for identifying carcinogens. *Toxicol Pathol. 1998 Jul-Aug;26(4):461-73.*	--	--	Yes	No
--	European Chemicals Agency (ECHA)	2012	Guidance on the Application of the CLP Criteria. *Version 3.0 November 2012*	--	--	Yes	No
--	European Commission, editor Joint Research Center, Institute for Health and Consumer Protection	2003	TGD for Risk Assessment (2003): Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances and Commission Directive 98/8/EEC concerning the Placing of Biocidal Products on the market.	--	No	Yes	No
--	Marsman D. S. *et al.*	1995	Chronic nephropathy and renal carcinogenicity of o-benzyl-p-chlorophenol in F344/N rats and B6C3F1 mice. *Fundam Appl Toxicol. 1995 Sep;27(2):252-62.*	National Institute of Environmental Health Services, Research Triangle Park, North Carolina, USA	--	Yes	No
--	Pritchard J. B. *et al.*	2003	The Role of Transgenic Mouse Models in Carcinogen Identification. *Environ Health Perspect 2003 111(4): doi:10.1289/ehp.5778*	--	--	Yes	No
A3.1(01) A3.10(01) A3.13(01)	Jungheim, R.	2007a	Physicochemical properties of chlorophene.	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany	Yes	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
A3.2(01)	Olf, G.	2006	Vapor pressure, physical-chemical properties.	Bayer AG, BTS-PT-RPT-KPM, Leverkusen, Germany	Yes	No	Yes
A3.2(02)	Beiell, U.	2007	Calculation of Henry's Law Constant of Chlorophen (2-benzyl-4-chlorophenol).	Dr. Knoell Consult GmbH, Leverkusen, Germany	No	No	Yes
A3.3(01)	Kraus, H.	2006a	2-Benzyl-4-chlorophenol / Appearance.	LANXESS Deutschland GmbH, Leverkusen, Germany	No	No	Yes
A3.4(01)	Jungheim, R.	2007b	Spectral data of chlorophene.	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany	Yes	No	Yes
A3.5(01)	Jungheim, R.	2006a	Determination of the water solubility (flask method) of chlorophene at 10 °C, 20 °C and 30 °C.	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany	Yes	No	Yes
A3.5(02) A3.9(03)	Erstling, K.	2002	Water solubility, Preventol O extra in Schuppen.	Bayer AG, ZF-Zentrale Analytik, Leverkusen, Germany	Yes	No	Yes
A3.6(01), A3.9(01)	Greenwood, J.	2003a	BCP: Determination of the partition coefficient.	Covance LaboratoriesLtd., North Yorkshire, England	Yes	No	Yes
A3.7(01)	Jungheim, R.	2007c	Solubility of chlorophene in methanol and toluene at 10 °C, 20 °C and 30 °C.	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany	Yes	No	Yes
A3.9(04)	Jungheim, R.	2004	Solubility of Preventol O extra in organic	Bayer Industry	Yes	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
			solvents.	Services, Leverkusen, Germany			
A3.9(02)	Feldhues, E.	2006	Statement Partition coefficient n-octanol / water of Preventol O extra, Temperature and pH dependence.	Bayer Industry Services, BIS-SUA-PUA I, Leverkusen, Germany	No	No	Yes
A3.11(01)	Heinz, U.	2007	Determination of safety-relevant data of Preventol BP.	Bayer Industry Services GmbH & Co. OHG, Safety / Environment / Analytics, Process and Plant Safety, Leverkusen, Germany	Yes	No	Yes
A3.17(01)	Kraus, H.	2006b	2-Benzyl-4-chlorophenol (chlorophene) / reactivity towards container material.	LANXESS Deutschland GmbH, Leverkusen, Germany	No	No	Yes
A4.1(01)	Erstling, K.	2007	Validation of a HPLC method for the determination of the relevant main and minor components in Preventol BP. CONFIDENTIAL	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany	Yes	No	Yes
A4.2(02)	Meinerling, M.	2007a	Validation of an analytical method for the determination of Preventol BP (chlorophene) in water.	Institut für Biologische Anlaytik und Consulting IBACON GmbH, Rossdorf, Germany	Yes	No	Yes
A5.3.1(01)	Kugler, M.	2003	Determination of the antimicrobial effects of Preventol BP against bacteria and fungi.	Bayer Chemicals AG, Leverkusen, Germany	No	No	Yes
A5.3.1(02)	Bomblies, L. and Wedde, A.	2000	Preventol BP (active substance). Determination of the “Minimal Inhibitory Concentration (MIC) against various test microorganisms.	Labor L+S, Bad-Bocklet-Großenbrach, Germany	No	No	Yes
A6_1_1	Confidential	1983a	Ortho-Benzyl Parachlorophenol, (Chlorophen):	Confidential	No	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
			Acute Oral Toxicity in the Rat.				
A6_1_2	Confidential	1983b	Ortho-Benzyl Parachlorophenol, (Chlorophen): Acute Percutaneous Toxicity in the Rat.	Confidential	No	No	Yes
A6_1_3	Confidential	1983c	Ortho-Benzyl Parachlorophenol, (Chlorophen): Acute Inhalation Toxicity in the Rat.	Confidential	No	No	Yes
(A6_1_4)	Confidential	1983	Preventol BP - Examination of its Irritative Effects on Skin and Mucosa.	Confidential	No	No	Yes
(A6_1_4)	Confidential	1983d	Ortho-Benzyl Parachlorophenol, (Chlorophen): Acute Dermal Irritation/Corrosivity in Rabbits.	Confidential	No	No	Yes
A6.1.4(1)	Confidential	2000	Primary Dermal Irritation Study in Rabbits with Preventol BP (EPA/OECD/MAFF Guidelines).	Confidential	Yes	No	Yes
A6_1_4(2)	Confidential	1983e	Ortho-Benzyl Parachlorophenol (Chlorophen): Acute Eye Irritation/Corrosion Test in Rabbits.	Confidential	No	No	Yes
A6.1.5	Confidential	2001	Dermal Sensitization Study in Guinea Pigs – Closed Patch Test Technique with Preventol BP (EPA/OECD/MAFF Guidelines)	Confidential	Yes	No	Yes
(A6_1_5)	Confidential	1986	Preventol BP - Test for sensitizing effect on guinea pig skin ("Open Epicutaneous Test" according to Klecak)	Confidential	Yes	No	Yes
(A6_1_5)	Confidential	2002	Preventol BP Schuppen - Study for the skin sensitiztation effect in guinea pigs (Buehler Patch Test)	Confidential	Yes	No	Yes
A6_2_(1)	Kao, L. R. and Birnbaum, L. S.	1986	Disposition of *o*-Benzyl-*p*-Chlorophenol in Male Rats	Systemic Toxicology Branch, NIEHS, Research Triangle Park, NC, USA	No	Yes	No
A6_2 (2)	Confidential	1994	Dermal Absorption of ^{14}C-*o*-Benzyl-*p*-Chlorophenol From a 5% Formulation.	Confidential	Yes	No	Yes
A6_2_6	Sonnex, T. S. and Rycroft R. J. G.	1986	Allergic Contact Dermatitis from Orthobenzyl P	St, John's Hospital for Diseases of the Skin,	No	Yes	No

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
			Chlorophenol in a Drinking Glass Cleaner	London, England			
A6_3_1	Confidential	1973a	21-Day Subacute Oral Toxicity Study with Santophen I in Beagle Dogs.	Confidential	No	No	No
A6_3_1	Sendelbach, L. E.	1982a	Repeated Oral Dose Study of o-Benzyl-p-Chlorophenol in F344/N Rats.	Battelle, Columbus, OH, USA	No	Yes	No
A6_3_1	Sendelbach, L. E.	1982b	Repeated Oral Dose Study of o-Benzyl-p-Chlorophenol in B6C3F_1 Mice.	Battelle, Columbus, OH, USA	No	Yes	No
A6_3_2	Confidential	1984	Ortho-Benzyl Parachlorophenol (Chlorophen): Preliminary Dermal Toxicity Study in the Rabbit.	Confidential	Yes	No	Yes
(A6_3_2)	Confidential	1989	Ortho-Benzyl Parachlorophenol (Chlorophen): 21-Day Percutaneous Toxicity Study in the Rabbit.	Confidential	Yes	No	Yes
A6_3_2(1)	Confidential	1985	Ortho-Benzyl Parachlorophenol (Chlorophen): 21-Day Dermal Toxicity Study in the Rabbit.	Confidential	Yes	No	Yes
A6_3_2(2)	Confidential	1985	Preventol BP-Subacute toxicological study in rabbits (3-week trial with cutaneous application)	Confidential	Yes	No	Yes
A6_4_1(1)	Birnbaum, L. S. *et al.*	1986	Prechronic toxicity of o-benzyl-p-chlorophenol in rats and mice. *Fundam Appl Toxicol. 1986 Nov;7(4):615-25.*	--	--	Yes	No
A6_5+6_7 A6_7	National Toxicology Program	1994	NTP Technical Report on the Toxicology and Carcinogenesis Studies of o-Benzyl-p-Chlorophenol (CAS No. 120-32-1) in F344/N Rats and B6C3F1 Mice (Gavage Studies).	National Toxicology Program, Research Triangle Park, NC, USA	No	Yes	No
A6_4_1(2)	Confidential	1973b	90-Day Subacute Oral Toxicity Study with Santophen I in Beagle Dogs.	Confidential	No	No	No
A6_5	Confidential	2005	2-Benzyl-4-chlorphenol (Preventol BP) – Exploratory Subchronic Toxicity Study in Male Rats (16-Weeks Administration via Diet)	Confidential	No	No	Yes
A6_5+A6_7	Hejtmancik, M. *et al.*	1988a	The Chronic Gavage Study of o-Benzyl-p-	Battelle, Columbus,	No	Yes	No

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
			Chlorophenol (CAS No. 120-32-1) in Fischer 344 Rats.	OH, USA			
A6_6_1 (1)	Mortelmans, K. *et al.*	1986	*Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals.	EG&G Mason Research Institute & SRI International	No	Yes	No
A6_6_2	Confidential	1994	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells.	Confidential	Yes	No	Yes
A6_6_3 (1)	Confidential	2005	BCP: Mutation at the *hprt* locus of L5178Y Mouse Lymphoma Cells using the Microtitre® Fluctuation Technique.	Confidential	Yes	No	Yes
A6_6_3 (2)	Caspary, W. J. *et al.*	1988	The mutagenic activity of selected compounds at the TK locus: rodent vs. human cells.	–	No	Yes	No
A6_6_4 (1)	Confidential	1990	Nipacide BCP: Assessment of Clastogenc Action on Bone Marrow Erythrocytes in the Micronucleus Test.	Confidential	Yes	No	Yes
A6_6_4 (2)	Confidential	1972	Mutagenic Study with Santophen I in Albino Mice.	Confidential	No	No	No
A6_6_5	Confidential	2009	Single Cell Gel Electrophoreses (Comet) Assay in the Male Mouse: In Vivo.	Confidential	Yes	No	Yes
A6_7 (1)	Hejtmancik, M. *et al.*	1988b	The Chronic Gavage Study of o-Benzyl-p-Chlorophenol (CAS No. 120-32-1) in B6C3F_1 Mice.	Battelle, Columbus, OH, USA	No	Yes	No
A6_7_ (2)	Spalding, J. W. *et al.*	1999	Development of a transgenic mouse model for carcinogenesis bioassays: evaluation of chemically induced skin tumors in Tg.AC mice	Research Triangle Park, North Carolina, US	No	Yes	No
A6_7 (3)	National Toxicology Program	1995	One-year initiation/promotion study of *o*-Benzyl-*p*-Chlorophenol (CAS No. 120-32-1) in Swiss (CD-1®) Mice (Mouse Skin Study)	National Toxicology Program, Research Triangle Park, NC, USA	Yes	Yes	No
A6_8_1	Confidential	1984	Embryotoxicity (Including Teratogenicity) Study	Confidential	Yes	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
			with Preventol BP Technical in the Rat.				
A6_8_1	Confidential	1979	A Segment II Teratology Study with Santophen I in Rabbits	Confidential	No	No	Yes
A6_8_1_(1)	Confidential	1985a	Chlorophen: Teratology Study in the Rat.	Confidential	Yes	No	Yes
A6_8_1_(2)	Confidential	1985c	Chlorophen: Effects of Oral Administration upon Pregnancy in the Rabbit.	Confidential	Yes	No	Yes
A6_8_1_(4)	Confidential	1985b	Chlorophen: Effects of Oral Administration upon Pregnancy in the Rat. 1. Dosage Range-Finding Study.	Confidential	No	No	Yes
A6_8_1_(4)	Confidential	1985d	Chlorophen: Effects of Oral Administration upon Pregnancy in the Rabbit. Dosage range-finding study.	Confidential	No	No	Yes
A6.8.2(1)	Confidential	1973a	Reproduction Study with Santophen I in Albino Rats.	Confidential	No	No	Yes
A6.8.2(2)	Confidential	1973b	Perinatal and Lactation Study with Santophen I in Albino Rats.	Confidential	No	No	Yes
A_6_8_2_(3)	Confidential	2008	Chlorophene: Two Generation Reproduction Toxicity Study by Gavage in Wistar Rats.	Confidential	Yes	No	Yes
(A6_10)	Kao, L. R., *et al.*	1986	Effect of *o*-Benzyl-*p*-Chlorophenol on Drug-Metabolizing Enzymes in Rats	Systemic Toxicology Branch, NIEHS, Research Triangle Park, NC, USA	No	Yes	No
A6_12_1	Confidential	2007	Medical statement – 2-benzyl-4-chlorophenol (BP)	Confidential	No	No	Yes
A7.1.1.1.1(01)	Greenwood, J.	2003b	BCP: Evaluation of hydrolysis as a function of pH (HPLC screen).	Covance LaboratoriesLtd., North Yorkshire, England	Yes	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
A7.1.1.1.2	Meinerling, M. and Herrmann, S.	2007	Phototransformation of Preventol BP (Chlorophene) in Water.	Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany	Yes	No	Yes
A7.1.1.1.2	Meinerling, M.	2011	Non-GLP Statement on IBACON Project 33341176, Photolytic degradation of Preventol BP	Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany	--	No	Yes
A7.1.1.1.2	Freudenberg, Ch. and Wesener, J. R.	2011	Structure elucidation of the major photolysis product of Preventol BP (chlorophene)	Currenta GmbH & Co. OHG, Leverkusen, Germany	Yes	No	Yes
A7.1.1.2.1(01)	Bealing, D. J. and Watson, S.	2002	BCP: Assessment of ready biodegradability by measurement of carbon dioxide evolution.	Covance Laboratories Ltd., Harrogate, England.	Yes	No	Yes
A7.1.1.2.1(02)	Reis, K. H.	2007a	Ready biodegradability of chlorophene in a manometric test	Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany.	Yes	No	Yes
A7.1.1.2.1(03)	Swisher, R. D. and Gledhill, W. E.	1973	Microbial degradation of O-Benzyl-p-Chloro-phenol *CSMA – Proceedings of the 60th Annual Meeting*	Published by Chemical Specialities Manufacturers Association Inc.	--	Yes	No
A7.1.1.2.2(01)	Reis, K. H.	2007c	Inherent Biodegradability of Chlorophene in a Zahn-Wellens/EMPA Test.	Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany.	Yes	No	Yes
A7.1.2.1.2	Reis, K. H.	2007b	Anaerobic biodegradability of Chlorophene in digested sludge: Measurement of gas production.	Institut für Biologische Analytik und	Yes	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
				Consulting IBACON GmbH, Rossdorf, Germany.			
A7.1.2.1.2	Gerhardz, T.	2011	Biodegradation of 5 mg/kg Preventol BP® (2-benzyl-4-chlorophenol) in pork liquid manure under anaerobic conditions.	Lanxess Deutschland GmbH, Leverkusen.	No	No	Yes
A7.1.3(01)	Jungheim, R.	2006b	Determination of the Adsorption Coefficient (K_{oc}) by High Performance Liquid Chromatography (HPLC) Method of Chlorophene.	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, Leverkusen, Germany	Yes	No	Yes
A7.2.1	Nitsche, M.	2011	Biodegradation of Preventol® BP (Chlorophen) in soil under aerobic conditions	LANXESS Deutschland GmbH, Leverkusen, Germany	No	No	Yes
A7.2.3.1(01)	Meinerling, M.	2007b	Determination of the Adsorption / Desorption Behaviour of 2-Benzyl-4-chlorophenol (Preventol BP).	Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany	Yes	No	Yes
A7.3.1(01)	Fàbregas, E.	2006	Calculation of indirect photodegradation of Chlorophene.	DR. KNOELL CONSULT GmbH, Mannheim, Germany	No	No	Yes
A7.4.1.1(01)	Confidential	1986	Preventol BP (2-benzyl-4-chlorophenol): Fish Toxicity, *Brachydanio rerio*.	Confidential	No	No	Yes
A7.4.1.2(01)	Caspers, N.	1986a	Preventol BP (2-benzyl-4-chlorophenol): Toxicity, *Daphnia magna*.	Bayer AG, WV Umweltschutz, Leverkusen, Germany	No	No	Yes
A7.4.1.3(02)	Caspers, N.	1986b	Preventol BP (2-benzyl-4-chlorophenol): Growth inhibition test Algae.	Bayer AG, WV Umweltschutz, Leverkusen, Germany	No	No	Yes
A7.4.1.3	Egeler, Ph. *et al.*.	2006	Preventol BP: A study on the toxicity to algae	ECT Ökotoxikologie GmbH, Flörsheim,	Yes	No	Yes

Section No. in in CA report on chlorophene	Author(s)	Year	Title	Testing Company	GLP Study (Yes/No)	Published (Yes/No)	Data Protection Claimed (Yes/No)
			(*Pseudokirchnerella subcapitata*)	Germany and Batelle, Geneva, CH			
A7.4.1.4	Caspers, N. and Müller, G.	1991	Untersuchungen zur Bakterientoxizität von Preventol BP Schuppen.	Bayer AG, Institut für Umweltanalyse und Bewertungen, Leverkusen, Germany	Yes	No	Yes
A7.4.2(01)	Fàbregas, E.	2007	Calculation of the Bioconcentration Factor (BCF) of Chlorophene.	DR. KNOELL CONSULT GmbH, Mannheim, Germany	No	No	Yes
A7.4.3.2(01)	Confidential	2008	Toxicity of 2-Benzyl-4-chlorophenol (Preventol BP) to Zebra-fish (*Danio rerio*) in an Early-Life Stage Test	Confidential	Yes	No	Yes
A7.4.3.3.1	Confidential	2009	Bioconcentration: Flow-through Fish Test with Chlorophene (Preventol BP)	Confidential	Yes	No	Yes
A7.4.3.4(01)	Weyers, A.	2007	Chlorophene, *Daphnia magna* Reproduction Test.	Bayer Industry Services GmbH& Co: OHG, Leverkusen Germany	Yes	No	Yes

8 ANNEXES (EXCERPTS OF DOCUMENT III-A TO THE ACTIVE SUBSTANCE DOSSIER)

Annexed to the CLH report are study summaries of relevant health and environmental studies in the form of excerpts of Document III-A from the active substance dossier submitted under the Biocidal Products Directive (98/8/EC). The annexes are confidential.

List of annexes

Annex 1 Extract from Competent Authority Report – Directive 98/8/EC on the placing of biocidal products on the market.

Document III-A (A6.1-A6.12), study summaries – active substance, section A6: human health

Annex 2 Extract from Competent Authority Report – Directive 98/8/EC on the placing of biocidal products on the market.
Document III-A (A7.1-A7.4), study summaries – active substance, section A7: environment