Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Chlorophene

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

Adopted

12 March 2015

CLH-O-0000001412-86-58/F

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

12 March 2015

CLH-O-0000001412-86-58/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonized classification and labelling (CLH) of:

Chemical name: **chlorophene**

EC Number: **204-385-8**

CAS Number: **120-32-1**

The proposal was submitted by **Norway** and received by RAC on **27 September 2013**.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Norway has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **09 September 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions on **24 October 2014**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-rapporteur, appointed by RAC: **Katalin Gruiz**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonized classification and labelling was adopted on **12 March 2015** by consensus.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal		chlorophene; 2-benzyl-4-chlorophenol	204-385-8	120-32-1	Carc. 2 Repr. 2 Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1A Eye Dam. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H332 H315 H317 H318 H372 (kidney) H400 H410	GHS08 GHS07 GHS09 Wng	H351 H361f H331 H315 H317 H318 H372 (kidney) H410		M=1 M=100	
RAC opinion		chlorophene; 2-benzyl-4-chlorophenol	204-385-8	120-32-1	Carc. 2 Repr. 2 Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H332 H315 H317 H318 H373 (kidney) H400 H410	GHS08 GHS05 GHS07 GHS09 Dgr	H351 H361f H332 H315 H317 H318 H373 (kidney) H410		M=1 M=100	
Resulting Annex VI entry if agreed by COM		chlorophene; 2-benzyl-4-chlorophenol	204-385-8	120-32-1	Carc. 2 Repr. 2 Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H332 H315 H317 H318 H373 (kidney) H400 H410	GHS08 GHS05 GHS07 GHS09 Dgr	H351 H361f H332 H315 H317 H318 H373 (kidney) H410		M=1 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

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.

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)**.

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.

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).

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

According to the DS, chlorophene caused serious eye damage in albino rabbits. Lesions of the cornea and iris as well as conjunctival redness and chemosis were observed, all of which persisted until the end of the observation period. The DS proposed to classify chlorophene as Eye Dam. 1 (H318) on the basis of severe ocular irritation which persisted and became more marked at each subsequent examination. At termination (72 h after treatment), the effects were not expected to reverse.

Comments received during public consultation

Three MSCA and a manufacturer of chlorophene agreed with the classification proposal. One MSCA requested that the DS specify the classification criteria in CLP for which the classification for Eye Dam. 1 was proposed.

Assessment and comparison with the classification criteria

Two studies were summarised in the dossier. The first study (dated 1983) followed a method similar to OECD TG 405 but was non-GLP. Chlorophene produced ocular irritation in rabbits characterised by diffuse opacity or translucency of the whole visible corneal surface, injection of the conjunctival blood vessels and eversion of the eye lids due to moderate chemosis in all animals. The eye irritation scores are shown in the table under "Supplemental information - In depth analyses by RAC" (BD; Annex 2). Irritation responses became more marked as the study progressed which led to termination of the experiment after 72 h, as reversibility was not expected.

According to the CLP criteria, 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 within 21 days, then it should be classified for serious eye damage in category 1.

The second study (also dated 1983) was not carried out according to OECD test guidelines or GLP. The results suggested that the eye effects due to chlorophene are less severe and also reversible. Average scores for irritation to the cornea, iris, conjunctiva (redness/chemosis) were 1.50, 1.33, 2.17/2.00 respectively.

RAC concludes that the findings described in the first study were more reliable. Given that the criteria for Eye Dam. 1 specifies that irreversible effects need only occur in one animal to warrant classification, **RAC agrees with the DS that the data from this study is sufficient to classify chlorophene with Eye Dam. 1 (H318).**

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.

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).

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).

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).**

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.

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).

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.

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.

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.

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.

ENVIRONMENTAL HAZARD ASSESSMENT

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.**

Additional references

Andersen KE and Maibach HI, 1980. Cumulative irritancy in the guinea pig from low grade irritant vehicles and the angry skin syndrome. Contact Dermatitis 6, 430-43.4

EU TGD (2003) 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 and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part III. European Commission Joint Research Centre.

Verhaar, HJM; Mulder, W. and Hermens, JLM (1995) QSARs for ecotoxicity. In: Overview of Structure-Activity Relationships for Environmental Endpoints. Part 2. Description of selected models.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC comments (excluding confidential information).