Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CLOROFENE; CHLOROPHENE; CLOROPHENE; 2-BENZYL-4-CHLOROPHENOL

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: clorofene; chlorophene; clorophene; 2-benzyl-4-chlorophenol
CAS number: 120-32-1
EC number: 204-385-8
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2014	France		MemberState	1
Comment received				
MS-FR agrees with the proposed classification for health hazard. Please, would you be more accurate on which criteria for classification with eye damage are fulfilled. MS-FR agrees with the proposed classification for environment: H400 and H410; We also agree with the chronic M-factor of 100. Nevertheless, more acute toxicity data on fish are needed to confirm the acute M-factor of 1.				
Dossier Submitter's Response				
Thank you for your support regarding the proposed classification for health hazard. Regarding the criteria for classification with eye damage, please see our response in comment number 20. Thank you for stating your agreements with the proposed environmental classification of H400 and H410 with a chronic M-factor of 100. Regarding your comment on the acute M-factor, please see our response to comments number 15 and 32.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	2
Comment received				
General comments • NL agrees with the proposed classification for chloroprene. • We have some questions regarding repeated dose toxicity, skin irritation and reproductive toxicity. • NL agrees with the conclusion that the substance cannot be considered readily biodegradable but does not share the dossier submitters reasoning that the results of the ready biodegradability tests are ambiguous. • We cannot support the conclusion on bioaccumulation as relevant study details are missing.				
Dossier Submitter's Response				
Thank you for your comments and support. Regarding the questions on repeated dose				

toxicity, skin irritation and reproductive toxicity please see our response to comment number 28, 18 and 12. Regarding the comments on biodegradation and bioaccumulation, please see our response to comment number 30.
RAC's response
Noted. We appreciate NLs clarification on biodegradability. Please see the detailed explanation in comment 30.

Date	Country	Organisation	Type of Organisation	Comment number
07.10.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	3
Comment received				
The following comments are related only to the classification derived from ecotoxicological and env. fate data. Comments on C&L for toxicological endpoints are filed separately. For the ecotoxicological and env. fate endpoints Lanxess supports the following classification and labelling of chlorophene: Aquatic Acute 1; H400 Aquatic Acute M-factor=1 Aquatic Chronic 1; H410 Aquatic Chronic M-factor=10 This classification proposal, where differing from the applicant's proposal, are supported by study data and sound scientific arguments in the related subsections of the commenting form. ***ECHA note: The following attachment was provided [Attachment 1]*** Chlorophene - Comments on the dossier proposing harmonised classification and labelling submitted by Norway (Confidential attachment).				
Dossier Submitter's Response				
Thank you for your comment. Please see our response to comment no. 31.				
RAC's response				
RAC's view is that the chronic fish test is valid and its result of 0.58 µg/L justifies M-factor of 100 (see comment and responses under No. 30 and 31).				

Date	Country	Organisation	Type of Organisation	Comment number
25.09.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	4
Comment received				
The following comments are related to Human Health Classification only. Comments on C&L for ecotoxicological endpoints are filed separately. For Human Health Lanxess supports the following classification and labelling of chlorophene: Acute Tox 4 (H332); Skin Irrit 2 (H315); Skin Sens. 1B (H317); Eye Dam 1 (H318);				

STOT RE 2 (H373); This classification proposal, where differing from the applicant's proposal, is supported by additional study data and sound scientific arguments in the related subsections of the commenting form. The Lanxess proposal is also attached to the web pabe as comprehensive document. ***ECHA note: The following attachment was provided [Attachment 2]*** Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section. (*The content of this attachment, except for the References on the last two pages, is already available in this comments table*)
Dossier Submitter's Response
Thank you for your comment. Please see our response to comment number 9, 13, 24 and 27 (referring to 28).
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2014	Germany		MemberState	5
Comment received				
The German CA agrees with the proposed classification for acute toxicity, skin irritation, skin sensitisation, eye damage and carcinogenicity. The proposed classification for reproductive toxicity and specific target organ toxicity is not supported. We agree with the proposed classification as H400 and H410, however the M-factor for acute aquatic toxicity might be changed to the chronic factor.				
Dossier Submitter's Response				
Thank you for your comments. Regarding the classification for reproductive toxicity and specific target organ toxicity you wil find our response in comments number 14 an 28. Regarding the M-factor for environmental classification, please see our response to comment no. 32 (under the sub-heading "Acute aquatic toxicity").				
RAC's response				
Noted. The value of acute and chronic M-factor may differ from each-other and may be based on results acquired from different test-organisms.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2014	France		MemberState	6
Comment received				
4.8.1 Summary and discussions of carcinogenicity We are not certain that it is relevant to refer to another dossier in the core dossier.				
Dossier Submitter's Response				
Thank you for your comment. The referral to another dossier was made since the compound evaluated in the dossier referred was classified as a carcinogen and the data involved cancer development at and above MTD and there was limited data on genotoxicity. Referral				

to previous evaluations of other compounds with respect to carcinogenicity was judged as relevant.
RAC's response
We agree with the Dossier Submitter.

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	7
Comment received				
The Netherlands agrees with the classification of Carc. 2 (H351) based on the renal tubule combined adenoma/carcinomas in B6C3F1 male mice and the renal transitional cell carcinomas in F344/N female rats. We agree that renal transitional epithelium carcinomas are rare but it is incorrect that this type of tumour has not been recorded in NTP historical control data (Table 22 in p.47 and p.52, CLH). A study by Haseman et al. (1998) reports an incidence of renal transitional epithelium carcinomas of 1 out of 1348 (0.07%) in female rats. In addition, Chandra et al. (1993) report a spontaneous incidence of renal transitional cell carcinoma of 0.09% in F344 rats. A similar correction can be made under table 24 (p.49, CLH) in point 'e' which states that the historical control for renal tubule carcinomas was 0/949. Haseman et al. (1998) report a rate of 1 in 1351 (0.07%) renal tubule carcinomas in male B6C3F1 mice.				
Dossier Submitter's Response				
Thank you for your comments. The details concerning historical background numbers have been elaborated. For response to comments, please see the response to comment number 9 for details. The historical background incidences depends on the strain used, forage given and other conditions, hence the most appropriate background levels for the two NTP-studies are the NTP background levels now described in more detail in response to comment number 9. For clarity, in Haseman et al., (1998) the studies were carried out within a time window of approximately seven years (last entry date 010197). The report includes all feeding studies without exact specification of route included. In the NTP report the background numbers given are those available relative to the time point when the study was conducted (dated 20. August 1992 in the original study report). In Chandra et al., (1993) the incidence and histopathological features of various long-term developing renal tumors observed in control rats from 10 carcinogenicity studies (530 males and 530 females) in F-344 rats from Charles River Breeding Laboratories are reported. No transitional cell carcinomas were reported among 530 females F-344 rats whereas they report on one case of TCC among 530 F-344 males. In the original NTP report historical background numbers dated 20. August 1992 taken from the NTP-database for renal tubule carcinomas was 0/949 as given in point 'e' in Table 24 in the CLH-report.				
RAC's response				
The additional details were helpful.				

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Finland		MemberState	8
Comment received				
We think that the data presented in the CLH report may warrant classification for Carc 2 according to Regulation (EC) No 1272/2008 (CLP). However, at present, the reporting of the				

data in the dossier is not sufficient and there are too many inaccuracies to enable final judgement. In general, we think that the proposal would benefit from more accurate and comprehensive reporting and justification throughout.
The proposed classification for carcinogenicity is based on tumour findings in three separate studies. Incidence of rare renal transitional cell carcinomas in two female rats in a two-year gavage study is stated as equivocal evidence of carcinogenic effect. According to dossier submitter the historical incidence of this tumour is not reported in the NTP historical control data (the historical incidence is 0/1068), and thus this tumour type is very rare. Yet, it is not stated whether any other historical control data is available. Since historical control data is very important in judgement of biological significance of rare tumours occurring at low incidence, this information is essential. In general we agree with the dossier submitter that incidence of these rare tumours is relevant for classification for carcinogenicity.
In contrast, we consider the relevance of tumours observed in the two other key studies obscure. In a two-year gavage study the incidences of renal tubule adenoma and carcinoma increased significantly in male mice. This study seems to suffer confounding effect of excessive toxicity, since according to body weight reductions, maximum tolerated dose is exceeded even at the lowest dose of chlorophene tested (120 mg/kg) in males. Survival of the animals is also severely effected in this study. Moreover, there are several inaccuracies in reporting which raise questions. For example, at page 51 it is stated: ”liver and kidney weights were dese-dependently increased in both sexes…”. However, according to the data presented in table 25, absolute kidney weights were significantly decreased at all doses in both sexes and absolute liver weights were significantly decreased in males. The only significant increases are relative liver weights at highest doses in both sexes, presumably due to reductions in body weight.
In a 20-week dermal carcinogenicity study on Tg.AC mice significant carcinogenic effect on skin and significantly increased tumour multiplicity was observed. It is stated that only female mice and 13-20 animals per group were used in the study. The only results reported from this study are the skin tumour incidences, and that survival decreased in a dose dependent manner. We think that particularly since this is a non-guideline study, a more comprehensive reporting is required to assess whether the data is valid for classification.

Dossier Submitter’s Response

Thank you for your comments, and for pointing at obvious errors in the CLH-document. Due to cut-and-paste errors the sentence regarding liver and kidney weights are clearly not correct, and the referral to Figures/Tables are unclear.
In the chapter concerning Carcinogenesis the Tables are numbered 21-27, and in the text Tables 22-27 are sometimes referred to as Table 4.14 to 4.19. At the bottom of page 48 it is referred to Table 25, when it is meant to be Table 24.
In response to comment 9 we have elaborated on the important issue of historical background numbers for the rare renal transitional cell carcinomas, and on the issue of exceeding MTD in male mice.
With respect to the 20-week dermal carcinogenicity study on Tg.AC mice specific information concerning the study was included in the CLH-dossier at page 51 and 52, including table 27. The reduced survival observed was probably connected to the age of onset of treatment (18 weeks) and spontaneous development of odontomas leading to removal of mice from the study.

RAC’s response

The additional data were helpful.
Reporting and interpretation of the Tg.Ac mouse test was not optimal. This test was not informative about the carcinogenicity of chlorophene.

Date	Country	Organisation	Type of Organisation	Comment number
25.09.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	9

Comment received

4.7 Carcinogenicity

Lanxess disagrees with the Carc Cat 2 (H351) classification proposal. This proposal is based on equivocal evidence for renal transitional cell carcinoma in female rats (NTP TR 424, 1994, A6_5+A6_7), some evidence for renal tubule carcinoma in male mice (NTP TR 424, 1994, A6_7(1)) and a statistically significant effect in female transgenic mice after dermal exposure (Spalding et al., 1999).

Rather, Lanxess proposes non-classification for carcinogenicity for the following reasons:

NTP Study – 2-year gavage – rats (NTP TR 424, 1994, A6_5+A6_7):
In this chronic study, F344 rats received daily gavage administrations of 30, 60, and 120 (males) or 60, 120, 240 (females) mg/kg bw/day of chlorophene in corn oil. Treatment was continued for 2 years, with interim sacrifices at 13 and 65 weeks. The 2-year sacrifice comprised 50 animals per sex and dose, whereas each interim sacrifice comprised 10 animals per sex and dose.

In male rats no evidence of carcinogenic activity was seen, although kidney was the clear target organ for chlorophene toxicity. The most sensitive parameter of kidney toxicity was the severity of nephropathy, which increased dose dependently (severity grades 2.3, 2.8, 2.9, 3.3 for control, low-, mid-, and high-dose). Kidney weight was dose dependently enhanced in the mid- and high-dose group. Hyperplasia of renal tubules occurred in 17 of 50 males (control 3/50) and renal transitional cell hyperplasia was detected in 26 of 59 male rats (control 5/59, Marsmann et al., 1995) of the high-dose group (120 mg/kg bw/day). Thus, despite similar evidence of nephropathy in males and females and a higher severity of nephropathy in males, there were no increased incidences of neoplasms in male rats.

Female rats achieved chlorophene doses twice as high as male rats (0, 60, 120, and 240 mg/kg bw/day). The most sensitive parameters for kidney toxicity in female rats were increases in kidney weight and in severity of nephropathy, although severity grades were throughout lower than in males (1.2, 1.2, 1.5, 2.4 for control, low-, mid-, and high-dose). Distinct nephrotoxic effects were only seen in the highest dose group of 240 mg/kg bw/day, thus, female rats appeared to be less sensitive to chlorophene induced nephrotoxicity than male rats. Hyperplasia of renal tubules occurred only rarely in treated and untreated females, however, renal transitional cell hyperplasia was detected in 17 of 59 females (control 4/60, Marsman et al., 1995) of the high-dose group (240 mg/kg bw/day) as a component of some cases of severe nephropathy. One female each of the mid- and high-dose group were found to bear a renal transitional cell carcinoma (TCC). Since the spontaneous incidence of this tumour type in the collected NTP historical controls is very low, especially in female rats, and the incidences were not statistically significant, the finding was judged as equivocal evidence of carcinogenic activity. However, although given with 0/1068 in the NTP report, a meta-analysis of the available NTP control data for F344 rats fed NIH-07 diet within the relevant treatment period published on the NTP web-page http://ntp.niehs.nih.gov/?objectid=92E6AAA5-F1F6-975E-71C88528A3E7B315 shows that this tumour type has been recorded with 3/3334 for male and 2/3323 for female control animals of this strain. These control values are in line with 1 TCC/1348 female F344 rats in chronic feeding studies and 1 TCC/898 female F344 rats in chronic inhalation studies

published by Haseman et al (1998*). Thus, by comparison with historical control data it can be concluded that TCCs occur spontaneously, albeit rarely. Hence, a spontaneous occurrence of TCCs in chlorophene-treated animals cannot be dismissed. Therefore there is a need to evaluate whether from the substance specific data there is any indication that the observed tumours may be substance related.

• The negative result in the Comet assay (confidential, 2009, A6_6_5) together with the negative results in the mouse bone marrow micronucleus assay (oral route, confidential 1990, A6_6_4(1)) and in the Dominant Lethal Assay (intraperitoneal route, confidential 1972, A6_6_4(2)) show that chlorophene exerts no genotoxic activity in vivo.

• A typical phenomenon in carcinogenesis of non-genotoxic substances is progression from pre-neoplastic lesions to the malignant tumour. A review was conducted within the NTP study to specifically evaluate the occurrence of renal transitional cell hyperplasia, a potential precursor lesion for the development of transitional cell carcinoma (TCC). This review of high-dose and vehicle control rats from the 15-month interim and 2-year evaluation (the two time points are not differentiated in the report) was limited to the transitional epithelium lining, the renal pelvis, and papilla. An increased incidence of transitional cell hyperplasia was detected in 29% of the females dosed with 240 mg/kg bw/day (vehicle control, 4/60, 7%; 240 mg/kg, 17/59, 29%). However, hyperplasia of the transitional epithelium was also detected in male rats, and with 44% the frequency of hyperplasia was much higher (vehicle control, 5/59, 9%; 120 mg/kg, 26/59, 44%) – males showing higher incidences of hyperplasia than females at half the dose, whereas TCCs were not seen in male rats. Consequently, it is not plausible that the sporadically occurred TCCs in female rats are a consequence of transitional cell hyperplasia.

• In the urinary bladder transitional epithelium papilloma was observed in one female rat in the control group but in none animal of the treatment groups. Urinary bladder transitional epithelium hyperplasia was observed in one female in the control and one in the high-dose group. Male rats showed this hyperplasia in one animal in the control and the low-dose group each.

• Tumours or precursor lesions at the renal and bladder transitional epithelium did not occur in mice of either sex treated for up to 2 years with chlorophene.
Overall, there is no convincing picture of substance related tumour formation in the kidney of female rats. Although hyperplasia of the transitional epithelium occurs in treated rats, the gender dependency of hyperplasia and tumour occurrence is inversely related. Some spurious findings were seen in the transitional epithelium of the bladder in the control females. Mice did not show any proliferative lesions in the renal and bladder transitional epithelium. Therefore it is concluded that the sporadic occurrence of renal TCCs in female rats is not substance-related but considered as spontaneous lesions that does not support a classification of chlorophene as a Cat2 carcinogen (CLP).

* data have been submitted to the RMS Norway by Lanxess recently, i.e.
Haseman et al., 1998. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. Toxicologic Pathology 26, 428-441

NTP Study – 2-year gavage – mice (NTP TR 424, 1994, A6_7(1)):
In this chronic study, male and female B6C3F1 rats received daily gavage administrations of 120, 240, and 480 mg/kg bw/day of chlorophene in corn oil. Treatment was continued for 2 years, with interim sacrifices at 13 and 65 weeks. The 2-year sacrifice comprised 50

animals per sex and dose, whereas each interim sacrifice comprised 10 animals per sex and dose.

In female mice no evidence of carcinogenic activity was seen. The kidney was the clear target organ for chlorophene toxicity seen as dose dependently increased incidence and severity of nephropathy (2-year incidences: 19/50 for control to 50/52 for high-dose; 2-year severity grades: 0.4, 1.1, 1.8, and 2.2 for control, low-, mid-, and high-dose). Although prominent, evidence and severity of nephropathy was lower in female mice compared to male mice (see text below). No neoplasms were observed in female mice at all. Hyperplasia of renal tubule and transitional epithelium was not seen in female mice. General toxicity was evident in female mice as dose-dependently reduced body weight. Survival was generally low in female mice, with only 74% in the control group. Chlorophene treatment led to a survival rate of 51% in high-dosed females, i.e. to a survival decrease of > 30%. Body weight was reduced by 29% in the high-dose group. The significant and dose dependently increased occurrence of fibrous osteodystrophy in all treated groups (2/50, 20/50, 33/50, and 37/50 for the control, low-, mid-, and high-dose group) is ascribed to and correlated with the increasing severity of nephropathy and the development of secondary renal hyper-parathyroidism. All these results suggest that the MTD was exceeded in that study.

In male mice some evidence of carcinogenic activity was reported based on a significantly higher frequency of renal tubule adenomas in the high-dose group and of renal tubule adenomas and carcinomas combined in the mid- and high-dose group.
Renal tubule adenomas are benign tumours which were observed in the statistically significant frequency of 5/50 male mice of the high-dose group treated with 480 mg chlorophene/kg bw/day. Renal tubule carcinomas occurred, however, neither in a dose-dependent nor statistically significant manner. Two carcinomas were found in the mid-dose group and one carcinoma in the high-dose group. When combining carcinomas with adenomas the statistical significance is given again for the mid- and high-dose groups (6 of 50 each). Thus, enhanced tumour frequencies were seen in this study, however, it is questioned if this observation is adequate to classify the substance as carcinogen for the following reasons:

• Judging from the necropsy body weights at the 2-year sacrifice the MTD for male mice was already exceeded at the lowest dose tested. The terminal body weight of control and low-dose males was 48.0 and 39.1 g, respectively. This is a difference of -19%, whereas OECD TG 451 (in its current version adopted September 7, 2009) suggests a depression of body weight gain of around 10% as a suitable measure of evident toxicity for the highest dose groups. In the NTP study with chlorophene, the body weight depression in the mid-and high-dose groups were -26% and -32%, respectively, which certainly constitutes excessive toxicity.

• Nephropathy is a very common finding in senescent mice, especially in males. Thirty-nine out of 50 control males had minimal nephropathy at the 2-yr sacrifice. The incidence and severity of this lesion was enhanced by chlorophene (severity: 0.8, 2.0, 2.4, and 2.4 for control, low-, mid-, and high-dose). Nephropathy consisted of e.g. multifocal dilatation of renal tubules, tubule cell necrosis, and regeneration of tubule cells. As an apparent consequence, renal tubule hyperplasia was significantly increased, as well as the incidence of tubular adenoma (significant increase in the highest dose group only) but not of carcinoma. These findings became evident after two years of chlorophene administration in doses exceeding the MTD.
The fact that female mice did not develop renal tubule hyperplasia although nephropathy was apparent is not in conflict with the postulated correlation of hyperplasia with severity of

nephropathy in male mice. Senescent female mice suffer from nephropathy but this disease proceeds slower in female mice and consequently, incidence and severity are lower than in male mice.

• The low-dose group of 120 mg/kg bw/day shows sufficient reduction of body weight gain (-19%) and systemic toxicity (e.g. kidney weight reduced by 20%, nephropathy in 48 of 50 males) to qualify as an actual high-dose group (this was realized in the rat study). Two adenomas were recorded for males at this dose, which is no statistically significant frequency. Malign tumours did not occur at this sufficiently high dose. A historical control frequency of 4/949 (2-year gavage in corn oil) is given in the report for kidney tubule cell adenoma. A recently performed analysis of the NTP database for historical control data in studies with NIH-07 diet (5-year period through 1995) showed frequencies of 3/463 for adenomas in gavage (corn oil) studies. In one study with gavage (water) two renal cell carcinomas were observed in control males of a single study.

• The negative result in the Comet assay together with the negative results in the mouse bone marrow micronucleus assay (oral route) and in the Dominant Lethal Assay (intraperitoneal route) show that chlorophene exerts no genotoxic activity in vivo. Chlorophene clearly follows the typical mode of action for non-genotoxic acting substances – long-term exposure at elevated doses is required for the potential development of tumours. Thus, a threshold dose exists below which no effects should occur.
Based on the facts that 1) chlorophene is non-genotoxic in vivo, 2) tumours were only observed in male mice, in which chlorophene induced nephropathy is more pronounced than in female mice, 3) nephropathy is attended by necrosis, regeneration, hyperplasia, and adenoma formation in male renal tubules, 4) a significant increase was seen only for benign renal neoplasms, 5) carcinomas occurred only sporadically and neither in a dose-dependent nor significant manner, and 6) significantly enhanced tumour frequencies were seen only at doses exceeding the MTD, it is concluded that the 2-year study on mice does not support a classification of chlorophene as a Cat2 carcinogen (CLP). The data rather indicate that the tumours observed are a consequence of chlorophene induced nephrotoxicity in male mice at very high doses (240 to 480 mg/kg bw/day for 2 years) exceeding the MTD. This effect is fully covered by the proposed STOT RE2 classification for kidney effects and prevention of nephrotoxicity would also prevent of the consequences of nephrotoxicity. Based on the NTP studies and the review on toxicology of chlorophene of Stouten and Bessems (1998) other authors stated that ‘the mode of action dependent on regenerative hyperplasia will be expected to exhibit a threshold and not be relevant to humans at doses below those that cause nephropathy’ (Osimitz et al., 2013).

NTP Study - dermal – Swiss CD-1 mice (NTP TR444, 1995, A6_7(3)):
Groups of 50 male and 50 female Swiss CD-1 mice were topically exposed to chlorophene to study its effect as an initiator, promoter, and/or complete carcinogen. After initiation with a single topical dose of 10 mg chlorophene in acetone followed by repeated topical application of 0.1, 1, or 3 mg for 3 times a week over one year, chlorophene showed no carcinogenic activity. Additionally, chlorophene exerted no initiating activity in mice when chlorophene treatment was followed by topical application of the tumour promoter TPA (5 µg) over 6 months. Only in mice that were pre-initiated with DMBA (50 µg) and then topically treated for one year with 3 mg chlorophene, a weak tumour promoting activity became obvious. The incidence of papilloma was significantly enhanced in female mice. This activity, however, was much lower than that for DMBA/TPA treated mice and it was not seen after treatment with 0.1 or 1 mg chlorophene. Incidences of scaling and/or crusts, ulceration, and irritation in 3 mg chlorophene treated mice were much higher than the incidences of the DMBA/acetone control group. Because of the skin-irritating properties of chlorophene, an increased keratinocyte turnover is expected. In fact, hyperkeratosis was observed at 3

mg/mouse in a 3-week pilot study; over one year of continued exposure, hyperkeratosis increased in incidence and severity. Such a stimulation of cell proliferation may exert a promoting effect on initiated cells.
Chlorophene can be expected to be non-promoting at sub-irritating dermal concentrations. In the animal study, the irritation stimulus is exerted to the same skin area over one year on a daily basis. Due to the study design this has no correlation to any expected exposure pattern, the study is of limited relevance for classification as a carcinogen, as it is well known that chlorophene is a potent skin irritant and the observed promotion of skin tumours is a direct consequence of this property.
Histopathological investigations of kidney, liver, nose, and thymus did not reveal any chemical-related increased incidences of neoplasms or non-neoplastic lesions when chlorophene was topically applied as an initiator, a promoter, or as a complete carcinogen for up to one year.

Transgenic Mouse Model – dermal – female Tg.AC mice (Spalding et al., 1999, A6_7(2); Tennant et al., 1995):
Groups of 13-19 female Tg.AC mice, carrying an inducible v-Ha-ras oncogene were topically treated 3 times a week with 0.1, 1, or 3 mg chlorophene in acetone for 20 weeks, followed by a 6-10 week post-treatment time. The age of the animals at onset of treatment was 18 weeks, instead of the usual age of 7-8 weeks for this study. Dose range and frequency of treatment were the same as used in the initiation/promotion experiment of NTP (described above). No overt signs of skin irritation were recorded. Survival of mice was lower in the chlorophene treated groups, with 6 of 19 (32%) animals dying before the end of treatment time with 3 mg chlorophene. For a positive response papilloma were counted which at least persisted 3 weeks. Assessment was done by gross examination and no histopathological analysis was performed.

Animals treated with 3 mg chlorophene showed a significant increase of skin papilloma (84%) and a higher papilloma frequency per animal (2.3/animal) compared to the acetone control (29% papilloma, 0.3 papilloma/animal). However, the incidences were much lower than for the positive control TPA (1.25 µg), which induced 95% papilloma and the number of papilloma per animal was 19.5 in this group. Since tumour initiation is inherent in the transgenic genotype of Tg.AC mice, a positive result in the Tg.AC mice model represents a tumour promoting activity of the tested substance. Thus, the positive result for chlorophene in this assay reflects the results seen in the skin-painting study of NTP with chlorophene (NTP TR 444, see above) - a weak, much lower skin tumour promoting activity as for TPA can be postulated. And, as in the NTP mouse studies, only benign neoplasms developed. As chlorophene is a well-known skin irritant, although such effects were not described here, it can be postulated that the promotion of neoplasms is a consequence of regenerative proliferation. However, this assumption cannot be proven since histopathology was not performed in the transgenic mouse study.

The Tg.AC mouse model is not yet validated for regulatory purposes and its reliability can be questioned per se, but additionally several points are remarkable with regard to the reliability of this specific study:
1) no overt skin irritation or ulceration was determined by gross examination, although in the NTP skin paining study scaling and/or crusts, ulceration, and irritation was observed at the same dose and treatment regimen of chlorophene,
2) no histopathology was done, so that hyperkeratosis and other potential precursor of the papilloma could not be determined
3) survival of mice was decreased in the chlorophene treated groups, however, it has to be acknowledged that mice were 18 weeks old at onset of treatment, instead of 7-8 weeks - this led to an increase in spontaneously developed jaw tumours (odontomas start to occur

with 26 weeks of age in this strain); since any animals with odontomas were removed from the study this may have influenced survival (no specific information is given in the publication),
4) papilloma were seen to develop and regress over the course of a study – this reduces the significance of this assay as model for carcinogenesis,
5) it was highlighted by Ashby (1997) that disparate substances, including chlorophene, which affected different but only internal tissues in the standard rodent bioassay 'were seemingly metamorphosed into skin cacinogens in the Tg.AC model upon their application to the skin'. Chlorophene has never shown any effects to the skin in oral toxicity studies.
6) the reliability of this study was judged with 3 (not reliable) by the Norwegian Environmental Agency.

Overall, the Tg.AC mouse study shows a weak promoting activity of chlorophene for the development of benign neoplasms. No maligne tumours were induced. However, the study does not allow a final conclusion due to its insufficient reliability.

Conclusion for carcinogenicity
Although the findings in female rats and in male mice both involve the kidney at large, they are histologically completely unrelated and do therefore not corroborate each other. Whereas the adenomas in male mice affect the tubules with their simple cuboidal epithelium, the TCCs in the female rat affect the transitional epithelium of the renal pelvis which is histologically more related to the urinary bladder than to the renal tubules.

Although hyperplasia of the transitional epithelium occurs in treated female rats, the gender dependency of hyperplasia and tumour occurrence is inversely related. Some spurious findings were seen in the transitional epithelium of the bladder in the control females. Mice did not show any proliferative lesions in the renal and bladder transitional epithelium. Therefore it is concluded that the sporadic occurrence of renal TCCs in female rats is not substance-related but considered as spontaneous lesions.

In the kidney of male mice significantly enhanced adenoma frequencies were seen only at doses exceeding the MTD. The data indicate that the tumours observed are a consequence of chlorophene induced nephrotoxicity, followed by hyperplasia in male mice at very high doses (240 to 480 mg/kg bw/day for 2 years), a threshold-effect which is covered by the proposed STOT RE2 classification for kidney effects.

In a skin painting study on mice chlorophene was shown to exert a weak tumor promoting activity at a skin irritating dose which also induced hyperkeratosis. However, at sub-irritating dermal doses no promoting activity became obvious. In this study chlorophene showed no activity as tumour initiator or as complete carcinogen.

Finally, the Tg.AC transgenic mouse study shows a weak promoting activity of chlorophene for the development of benign neoplasms. No maligne tumours were induced. The absence of skin irritation at a dose which was shown to be irritating in other studies and the lack of histopathological investigations to determine proliferative precursor lesions as hyperkeratosis, amongst others renders this study not reliable. Therefore, this study should not be taken for classification of chlorophene.

Overall, based on the in-depth evaluation of the data from long-term animal studies and taking into consideration that chlorophene is non-genotoxic, it is concluded that classification of chlorophene as a Cat2 (CLP) carcinogen is not justified. Rather, the effects can be seen as consequence of the properties of chlorophene as kidney toxicant (kidney adenoma in male mice) and as skin irritant (papilloma after dermal treatment). Since

chlorophene was shown to be non-genotoxic in vivo, a clear threshold for nephrotoxicity can be postulated (see comments to chapter 4.6 Repeated Dose Toxicity). Thus, protection from nephrotoxicity (see the proposed STOT RE2 classification for kidney effects) and skin contact (see the proposed classification for skin irritation with Skin Irrit Cat 2 and for skin sensitization with Skin Sens Cat 1B) will adequately protect from subsequent damage.

ECHA note: The following attachment was provided [Attachment 2]

Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section.
(*The content of this attachment, except for the References on the last two pages, is already available in this comments table*)

Dossier Submitter's Response

Thank you for your comments.

We respond to the comments in the order they appear in the comments:
NTP Study – 2-year gavage – rats (NTP TR 424, 1994, A6_5+A6_7)
NTP Study – 2-year gavage – mice (NTP TR 424, 1994, A6_7(1))
NTP Study - dermal – Swiss CD-1 mice (NTP TR444, 1995, A6_7(3))
Transgenic Mouse Model – dermal – female Tg.AC mice (Spalding et al., 1999, A6_7(2); Tennant et al., 1995)

NTP Study – 2-year gavage – rats (NTP TR 424, 1994, A6_5+A6_7)
The most appropriate and important comparison of an experimental group is with its concurrent control. Moreover historical background data provide relevant data for interpretation of results, especially with respect to rare neoplasms. In the dossier, historical background data from similar NTP-studies (2-year F344 rat gavage studies fed the NIH-07 diet, and given corn oil) was described (0/400, dated 20. August 1992 in original study), whereas a broader description of other relevant availiable historical reference data is clearly beneficial for the evaluation due to the rare tumors in the chlorophene-exposed female rats. Very few cases of TCC have been described in historical control data. Only two cases of TCC had been observed among 3474 control females, with none among chronic gavage studies, summarised from all available NTP control data for F344 rats fed the NIH-07 diet published on the NTP web-page
(http://ntp.niehs.nih.gov/results/dbsearch/historical/nih07/index.html#4).
As summarised in Haseman et al., 1998 of studies carried out within a time window of approximately seven years (last entry date 010197) at NTP, there had been only one case of TCC among 1348 females in chronic feeding studies, and only one case of TCC among 898 females in chamber studies. Exact routes of exposure are not given in this summary.
In another study, confirming the rarity of TCC in F344 female rats, Chandra et al., (1993) reported on the incidence and histopathological features of various long-term developing renal tumors observed in control rats from 10 carcinogenicity studies (530 males and 530 females) in F344 rats from Charles River Breeding Laboratories. No transitional cell carcinomas were reported among 530 F344 females.
Hence, it is highly unlikely that two cases of TCC occur by chance in the chlorophene NTP study (NTP TR 424).

In the CLH-report it was concluded that several of the key studies exhibit study insufficiencies that hamper establishment of solid conclusions on genotoxicity, but based on an overall evaluation of the available data using a Weight of Evidence approach the decision on the genotoxicity is negative, and no classification was proposed. In particular, an *in vivo* comet assay was conducted by the applicant, as agreed upon during the evaluation process,

due to indications of genotoxic effects and study inconsistencies in the exisiting data on genotoxicity. The assessment of genotoxic effect in the target organ, the kidney, in this study was not performed. Hence no substantiated conclusion on genotoxicity could be made for the target organ and the *in vivo* comet results were rendered of less importance in the evaluation of chlorophene.
In Hard et al., 1998 in an article concerning mechanisms of chemically induced renal carcinogenesis in the laboratory rodent, it is stated that "In contrast to the classical experimental models, many of the chemicals testing positive for the kidney in the conventional rodent bioassay are nongenotoxic" and suggesting the existence of "a range of diverse mechanisms underlying kidney carcinogenesis". These include direct and indirect acting genotoxic substances, prolonged stimulation of cell proliferation, or exacerbation of or interaction with the age related chronic progressive nephropathy. The latter two mechanisms involve low incidence and late occurring tumor development, as seems to be the case for chlorophene. Hard et al., 1998 report that 56 of 457 tested chemicals through NTP at the time of analyses were associated with increase in renal tumor incidence over control, and that 8 chemicals were associated with transitional cell tumors of the renal pelvis.

The consideration of the occurrence of pre-neoplastic lesions can be useful in the evaluation of whether the carcinogenic effect observed is substance related, depending on mode of action involved. For chlorophene the mode of action is not clear. It is important to keep in mind that the existence of hyperplasias *per se* is not on its own predicitive of the development of tumours. Hyperplasia may give rise to neoplastic changes, but neoplastic changes do occur without prior hyperplastic changes. This is an important distinction. Both males and females showed increased transitional cell hyperplasia in the 15-month interim evaluation and 2-year at the highest dose compared to controls. Thus, the observed tumours were accompanied by increases in pre-neoplastic lesions, but sex-differences seem to exist.

As stated in Haseman et al., 1998 " the evaluation of rare neoplasms may require less stringent statistical evidence in a given study if the low spontaneous can be demonstrated from an established historical control database", as is the case here. The finding of two cases of TCC among the chlorophene-treated animals should thus be considered as equivocal evidence of carcinogenicity.
Moreover, the tumour type (Transitional cell carcinoma) observed is relevant for humans, hence their occurrence should be included in the overall evaluation of the carcinogenicity of chlorophene.

<u>NTP Study – 2-year gavage – mice (NTP TR 424, 1994, A6_7(1))</u>
It is logical to speculate that the renal hyperplasias and neoplasms observed in male mice following orally administered chlorophene are secondary to the nephrotoxicity exerted by the test compound. As discussed in Marsman et al., 1995, previous studies in mice with other chemicals, both genotoxic and non-genotoxic (bromochloromethane CAS # 74-27-5; nitrilotriacetic acid CAS # 139-13-9; tri(2,3-dibromopropyl)phosphate CAS # 126-72-7; 2,4-diaminophenol dihydrochloride CAS # 137-09-7) showed poor association between nephropathy and renal carcinogenicity. Thus, although nephropathy may be a permissive factor, other primary and secondary mechanisms may be operative in the induction of the mouse renal neoplasms.
As stated in the CLH-report, the low-dose males express sufficient toxicity to qualify as an actual high-dose group. In the low-dose animals there is an increase in renal neoplasms, and in the mid- and high-dose the increase becomes statistically significant. Thus there appears to be a dose-response relationship between the dose of chlorophene and the development of renal neoplasms, starting at the lowest dose. Moreover, the increase in

renal tubule hyperplasia was significantly increased in the low-dose males. Other compounds have been classified as Category 2 carcinogens on the basis of tumours occurring at doses at and exceeding MTD, in which the incidence of tumours showed a dose-response relationship (e.g., Polyhexamethylene biguanide – PHMB, CAS # 27083-27-8). Similarly as with chlorophene, the data on genotoxicity were limited, and PHMB was concluded as non-genotoxic.
The benign kidney tubule cell adenomas have the potential to progress to malignant kidney tubule cell carcinomas and were considered along with the malignant tumours in the evaluation.

Altogether the dose-dependent increase in tumor incidence in male mice, despite exceeding the MTD, shoud be considered as support for a classification of the test compound in Category 2 (According to Regulation (EC) No 1272/2008 (CLP).

NTP Study - dermal – Swiss CD-1 mice (NTP TR444, 1995, A6_7(3))
The conclusion of this study is that chlorophene act as a weak skin tumour promoter under the experimental conditions used, and did not demonstrate activity as a skin tumour initiator or as a complete carcinogen. Chlorophene is irritating to the skin. However frequent and long term use potentialy leading to regenerative hyperplasia and subsequent weak skin tumour promoter activity should be considered.

Transgenic Mouse Model – dermal – female Tg.AC mice (Spalding et al., 1999, A6_7(2); Tennant et al., 1995)
The study in the transgenic initiated Tg.AC mice suggests that chlorophene is carcinogenic, most likely as a tumour promoter. Non-genotoxic carcinogens (epigenetic carcinogens) are often considered as tumour promoters, adding to the relevance of considering the two dermal studies (NTP and Spalding et al., 1999) as supportive of the carcinogenic effects observed in chronic gavage studies with rodents. The conclusion as carcinogenic in Tg.AC mice after dermal exposure was made although dose-dependent decreased survival was observed at 20 weeks. The latter was probably connected to the age of onset of treatment (18 weeks) and spontaneous development of odontomas leading to removal from study. Dermal dosing of female Tg.AC mice with 3 mg chlorophene in acetone over 20 weeks led to a significant increase of skin tumours (84% of animals had tumours) compared to the acetone control (29%). In this model papillomas develop, with potential to further progress into malignant tumors, hence considering the benign papillomas is relevant. The study was not considered highly reliable in a regulatory persepective, but we propose that the data is considered as supportive data for the evaluation of chlorophene as carcinogenic.

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

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

Conclusion on carcinogenicity

The reason for the proposal to classify chlorophene as Carcinogen category 2 as a weak non-genotoxic thresholded carcinogen with no clear mode of action identified is based on:

- The rare cases of renal transitional cell carcinomas in female rats (2-year study) in a well conducted study, not likely to occur by chance when comparing with historical reference incidences.
- The dose-dependent induction of renal neoplasms in male mice (2-year study) starting in the low dosed animals, despite exceeding the MTD.
- And supported by the the carcinogenic effect (as a weak tumour promoter) of chlorophene following dermal exposure in two dermal studies in female CD-1 mice or female Tg:AC mice.

References:

Chandra M1, Riley MG, Johnson DE. Spontaneous renal neoplasms in rats. J Appl Toxicol. 1993 Mar-Apr;13(2):109-16.

Hard GC. Mechanisms of chemically induced renal carcinogenesis in the laboratory rodent. Toxicol Pathol. 1998 Jan-Feb;26(1):104-12.

RAC's response

The additional data and perspectives provided by the Manufacturer and the Dossier Submitter were helpful. Details have been included in the RAC Opinion.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2014	Germany		MemberState	10

Comment received

We agree with the proposed classification for carcinogenicity Carc. 2, H351 based on the induced renal tubule adenoma and carcinoma in male mice (2-year study) along with the renal transitional cell carcinoma in female rats (2-year study).

Dossier Submitter's Response

Thank you for your comment and support.

RAC's response

Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	11

Comment received
The Netherlands agrees for no classification for mutagenicity.
Dossier Submitter's Response
Thank you for your comment and support.
RAC's response
Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	12

Comment received

The Netherlands agrees with the classification of Repro. 2 (H361f) based on reduced female fertility index. The reduced fertility was observed at dose levels inducing maternal toxicity including reduced body weight gain and kidney toxicity. It is unclear whether the effects on sexual function and fertility are secondary to the maternal toxicity. Although not mentioned, the androgen receptor binding ability of chlorophene at potencies similar to the CYP inhibition findings are both associated with delayed sexual development and decreases in reproductive performance (Martin et al. 2011).

The summary of a developmental study in NZW rabbits by CEPA, which may be different from the currently available studies, indicate results which were not mentioned in the CLH proposal and are considered relevant for classification.
A Developmental/Reproductive Toxicity study with chlorophene (97.9% pure) administered by gavage to mated New Zealand White rabbits (14-21/dose) on days 6-19 of gestation at 0 (corn oil), 10, 30 or 100 mg/kg showed a maternal NOEL > 100 mg/kg (No effects were reported at any dose.) and a developmental NOEL = 30 mg/kg (Possible adverse effects: an increased post-implantation loss and an increased incidence in ectopic kidney, ectopic testis and malformed kidney in fetuses at 100 mg/kg, when compared to concurrent and historical controls) (TOXNET (HSDB) and CEPA, 2000). Could you please confirm whether this study is present in the CLH proposal, confirm whether there is an increase in the described effects in this study and consider whether these effects warrant classification for developmental toxicity.

As no effect on the post-natal survival was observed in the 2-generation study at dose levels exceeding the levels applied in the supporting studies, we agree with no classification for effects on or via lactation.

Dossier Submitter's Response

Thank you for your comments and for highlighting additional data, and kindly providing us with the available data.

The U.S. Environmental Protection Agency's ToxCast research program uses high throughput screening (HTS) for profiling bioactivity and predicting the toxicity of large numbers of chemicals. ToxCast Phase I tested 309 well-characterized chemicals in more than 500 assays for a wide range of molecular targets and cellular responses. Of the 309 environmental chemicals in Phase I, 256 were linked to high-quality rat multigeneration reproductive toxicity studies in the relational Toxicity Reference Database (Martine et al.,

2011, Biology of Reproduction 85, 327–339). In this study Clorophene was included. Unfortunately, this study has not been included in the CLH report. But we would like to emphasize their findings in short: Based on *in vitro* testing clorophene was found to bind the androgen receptor and to inhibit CYP enzyme at similar potencies, which were both associated with delays in sexual development and decrements in reproductive performance. Clorophene was by the authors identified as a predicted reproductive toxicant.

Unfortunately, we were not able to provide the study report for the teratology study with rabbits by F.W.Ross within the given dealdline. The study is not included in the CLH report. However, we acknowledge that the study has been evaluated by the California EPA in 1995 as acceptable with possible adverse effects on development.

References:

MT Martin, TB Knudsen, DM Reif, KA Houck, RS Judson,
RJ Kavlock, DJ Dix, (2011) "Predictive Model of Rat Reproductive Toxicity from ToxCast High Throughput Screening" Biology of Reproduction, 85(2):327-339. 2011.

F.W. Ross, "Chlorophen: Effects of Oral Administration Upon Pregnancy in the Rabbit," (Supplement to LSR Report #: 85/BTP033/257; LSR Ltd., Suffolk, England; 10/7/92).

RAC's response

RAC noted the additional information, but this did not impact greatly on our interpretation of the original proposal.

Date	Country	Organisation	Type of Organisation	Comment number
25.09.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	13

Comment received

4.8 Toxicity for Reproduction

Lanxess agrees with non-classification for developmental toxicity.

Lanxess disagrees with the Repro Cat 2 (H361f) classification proposal of the Norwegian Environmental Agency. This proposal is based on statistically significant deviations from control reproduction parameters as fertility/fecundity index and oestrus cycle length in a two-generation reproductive toxicity study (OECD 416) with chlorophene doses of 60, 180, and 540 mg/kg bw/day (confidential 2008, A6_8_2(3)). Rather, Lanxess supports non-classification for reproductive toxicity for the following reasons:

P generation, Fertility index: In the P generation males the fertility index (insemination index) was 93.3% in the control group versus 100% in all treatment groups. The latter value is significantly higher. In the females of the P generation fertility indices of 93.3% (control), 86.7% (60 mg/kg bw/day), 86.7% (180 mg/kg bw/day) and 76.7% (540 mg/kg bw/day) were observed, only the high dose value being significantly lower compared to control. This lower fertility index in the highest dose group is considered as incidental finding by the author of the study as there were no changes observed in the related parameters, such as oestrous cyclicity and gross or microscopic findings of reproductive organs. Recently obtained historical control data of the lab (confidential, 2012*) show female fertility indices of 88 to 100% for P generation females (basis are 260 females of 9 two-generation studies). For a final conclusion on whether the slightly lower fertility index in high-dosed females is treatment releated or not, maternal toxicity should be considered as

a potential cause for the following reasons:
1) Although food consumption was increased by 24% in the 540 mg/kg bw/day females as well as to a lower extent in the 180 mg/kg bw/day (+ 10%) females during days 0-7 of gestation, this increased food intake did not result in an increase of body weight gain but, vice versa, to a reduced body weight gain. In the period of days 7-14 of gestation the body weight gain was reduced by 27% in the mid dose group females and by 31% in the high dose group females. This effect even increased in the high dose group to a body weight gain reduction of 41% during days 14-20 of gestation. Since neither litter weight nor litter size was significantly affected, this is a clear indication of maternal toxicity during pregnancy.
2) High dosed females showed strongly increased kidney weights (+33% absolute and +30% relative) and nephrotoxic findings at termination (22 out of 30 females with dilated tubules or nephropathy) as well as slightly increased liver weights (about +15%).
3) The observed LOAEL of 180 mg/kg bw/day for general toxicity in the two-generation study is well in line with the findings of a 90 day gavage study on rats (NTP TR 424, 1994, A6_4_1(1)), showing a treatment time close to the two-generation study. The LOAEL for general toxicity in females can be considered with 240 mg/kg bw/day in the 90 day study.
4) The litter size was not affected by treatment. If the fertilization of oocytes or the implantation of embryos were specifically impaired by chlorophene, one would expect not only a few dams not becoming pregnant but also pregnant dams delivering fewer offspring. This was not the case.
5) A dominant lethal test in mice with intraperitoneal injection of 100 and 200 mg/kg bw chlorophene did not affect fertilization, implantation, and early or late deaths (confidential, 1972, A6_6_4(2)) and the fertility index for males in the 2-generation study described above was 100% in the treatment groups.
In sum, the slightly lower fertility index (borderline response compared to historical control data) in the high dosed P females is interpreted as a consequence of maternal toxicity caused by chlorophene.

F1 generation, Fertility index: For the F1 generation females an unusually high fertility index of 100% was determined in the control and the lowest dose group (for comparison, the fertility indices in the P generation were 93.3% and 86.7% for these two groups, see above). As a consequence, the fertility index of 90% in the mid dose group and of 83.3% in the high dose group showed statistical significance although being within the historical control values of 80 to 100% for this type of studies in this laboratory (basis are 260 F1 females out of 9 two-generation studies, confidential, 2012*). No changes were observed in related histopathology of the reproductive organs of F1 females. Thus, the significance was due to the higher control value. However, maternal toxicity cannot be dismissed for the F1 females as shown below:
1) During gestation the body weight gain of F1 females was reduced by 14% in the mid dose group and by 19% in the high dose group (days 0-20). This effect was even more pronounced in the last week of pregnancy with a body weight gain reduction of 37% in the high dosed animals. Since neither litter weight nor litter size was significantly affected, this is a clear indication of maternal toxicity.
2) High dosed F1 females showed strongly increased kidney weights (+27% absolute and +30% relative) and nephrotoxic findings at termination (10 with nephropathy and 18 other findings out of 30 females) as well as slightly increased liver weights (about +10%).
In sum, the - compared to concurrent control - slightly lower fertility index in the mid and high dosed F1 females is interpreted as biological variability. Additionally, maternal toxicity was caused by chlorophene.

F1 generation, Fecundity index: For the F1 generation the high fecundity index of 100% was recorded for the control and the lowest dose group (for comparison, the fecundity indices in the P generation were 96.4% and 96.2% for these two groups). As a

consequence, the fecundity index of 96.0% in the high dose group F1 females showed statistical significance, although being within the historical control values of 86.7 to 100% for this type of studies in this laboratory (basis are 260 F1 females of 9 two-generation studies, confidential, 2012*). Treatment relation is excluded for this observation since the lower fecundity index was based on a single female with dystokia, a finding which was also seen in the control group of the P generation. Thus, the significance has to be considered as incidental and not treatment related.

F1 generation, Oestrous cycle length: The oestrous cycle length in the F1 females was 4.1 days (control), 4.0 days (60 mg/kg bw), 4.1 days (180 mg/kg bw), and 4.5 days (540 mg/kg bw), the latter being significantly higher compared to control. However, it has to be acknowledged that this higher value is comparable to the oestrous cycle length of the P generation females in this study, which was determined with 4.5 for control animals and 4.2, 4.5, and 4.5 for the increasing dose groups. Thus, the higher oestrous cycle length is not extended in the high-dosed F1 females but lower than usual in the control and low/mid-dose groups. Therefore, the oestrous cycle differences cannot be attributed to treatment but to biological variability. Additionally, no changes were observed in histopathology of the reproductive organs.

Additional information: No effects on fertility/fecundity were observed in an older one-generation reproduction toxicity study on rats with doses of up to 150 mg/kg bw/day (confidential, 1973a, A_6_8_2(1)). In this study no general toxicity was recorded for females. Additionally, several developmental toxicity studies on rats and rabbits do not show treatment related influences on litter size, resorptions or other parameters of female fertility.

In conclusion, the slightly lower fertility index of the high-dosed P generation females in a two-generation reproductive toxicity study (confidential, 2008, A6_8_2(3)) can be considered as secondary effect due to maternal toxicity of chlorophene in P and F1 females (body weight gain reduction of >>10% up to 41% in certain weeks of gestation – see description above, nephrotoxicity and its consequences). This toxicity of the parental generation is fully in line with the observations in repeated dose toxicity studies and is covered by the proposed STOT RE2 classification for kidney effects. The statistically significant deviations in the fertility index and oestrus cycle length/fecundity index of the F1 females can be regarded as biological variability, i.e. incidental findings being within the historical control values, but may also be due to the observed general systemic toxicity (nephropathy) in these animals. Overall the two generation reproductive toxicity study revealed no evidence of a specific reproductive potential of the test substance. Thus, no classification for reproductive toxicity/fertility is justified.
* data have been submitted to the RMS Norway by Lanxess recently, i.e.
Confidential, 2012. Historical Data, Issued on 2012-04-24. Related to the 'Two Generation Reproduction Toxicity Study in Wistar rats'.

ECHA note: The following attachment was provided [Attachment 2]

Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section.
(*The content of this attachment, except for the References on the last two pages, is already available in this comments table*)

Dossier Submitter's Response

Thank you for your comments. Below are respons to your comments.

The relevance of historical control data compared to concurrent control data in reproductive and developmental studies:
In Mylchreest and Harris paper from 2013 they highlight the importance of evaluating data from the exposed group primary with the concurrent control group: "Reproductive and developmental toxicity studies in laboratory animals are conducted as part of the process of evaluating the risk of pharmaceuticals and chemicals to human reproduction and development. In these studies, **comparison of data from groups dosed with the test article to a concurrent control group is considered the most relevant approach for the interpretation of adverse effect**s." (Mylchreest E and Harris SB, 2013, Methods Mol Biol. 2013; 947:275-94)

Comments to reduced fertility index in P and F1:
P generation: The fertility indexes for females in the P generation were 93.3% (control), 86.7% (60 mg/kg bw/day), 86.7% (180 mg/kg bw/day) and 76.7% (540 mg/kg bw/day). At the high dose the index was statistically significant lower compared to control. Recently obtained historical control data reported by Lanxess (confidential, 2012, uploaded in the confidential folder on CIRCABC as "HISTORICAL DATA REPRO STUDY 2012") show female fertility indices of 88 to 100% for P generation females (basis are 260 females of 9 two-generation studies), hence at highest dose the fertility index in this study is statistically significantly lower than both the concurrent control and the historical control group.

F1 generation: The fertility indexes for females in the F1 generation were 100% (control), 100% (60 mg/kg bw/day), 90% (180 mg/kg bw/day) and 83.3% (540 mg/kg bw/day). At the two highest doses the fertility indexes were statistically significant lower compared with the concurrent control group.

In conclusion; A statistically significantly reduced fertility index were observed in both P and F1 generation, for P generation the reduction was statistically significantly compared to both the concurrent control and the historical control group, and for the F1, the reduction was statistically significant compared to concurrent control.

Issues concerning maternal toxicity related to fertility:
As already stated in the CLH report, the Guidance on the Application of the CLP Criteria are discussing toxicity with respect to fertility as followed: **"Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes"**.
In this two-generation reproductive study the maternal bw gain at the highest dose in P and F1 generation were **less than 12% reduced compared to the control group (see table 1 below). No reduction in absolute body weight (the animals gained weight in all dose groups), no lethality or coma related to treatment was observed at any dose levels.** Hence, no marked systemic toxicity was observed according to the CLP.

The CLP follows this up by: "There is **no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity.** However, mating behavior can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behavior may not warrant classification (Guidance on the Application of the CLP Criteria, Version 4.0 – November 2013)".

Bodyweight gain:
Unfortunately we see that the calculation of body weight gain in table 29 in the CLP report is

incorrect. The calculation dose not takes into account that the starting body weight of the mice vary.

Body weight gain for week 0-10: In the study report the author states that for females there were no statistically significant differences in body weights and net weight gain between vehicle control and all the treated groups (the net body weight gain for week 1 to 10 for the exposed groups were within the range of 83-88g while for the control group the net gain was 80g in the same period of time).

Body weight gain during gestation: For the maternal body weights and weight change during gestation period the author states: "Treatment at 540 mg/kg Bwt/day resulted in significantly lower maternal mean body weights on day 20 and weight reduction during days 7-14 (dose-related), 14-20 and 0-20 of the gestation period when compared to the vehicle control. There were no treatment-related effects observed in maternal body weights and weight changes at 60 and 180 mg/kg Bwt during the different intervals of the gestation period when compared to the vehicle control except for lower weight changes during days 7-14 at 180 mg/kg Bwt".
We have calculated the percent maternal body weight gain (GD 0-20) and compared it to the control group in table 1.

The maternal body weight changes compared to the control was calculated as followed:
(Maternal bw at GD20/ maternal bw GD0) * 100% for all dose groups and control.
Then % bw control – % bw exposed group were performed to find the difference in bw gain.

Table 1: Percent maternal body weight gain (GD 0-20) compared to the control group.

Generation	60 mg/kg bw/day	180 mg/kg bw/day	540 mg/kg bw/day
P	-1 %	-3 %	-12%
F1	-1 %	-5%	-7%

In conclusion: Since the female fertility index was statistically significantly reduced in both generations compared to concurrent control and historical control data and there is no marked systemic toxicity observed, the reduced fertility index is likely to be treatment-related.

Limitation with the data for "Toxicity for reproduction"-endpoint was that several studies were non-GLP and performed before guideline. Individual data were missing and the purity of the test compound was not given. The studies were found to be of limited quality and not fulfilling the data requirement for classification. However, the two-generation study from 2008 submitted by the applicant follows the OECD 416 guideline, are GLP and the purity and stability of the test compound are given and is of good quality.

Comment concerning calculations of pre-implantation loss in two-generation study (Confidential, 2008 / A 6 8-2(3), uploaded in the confidential folder on CIRCABC as "TWO GEN REPRO STUDY 2008"):
We have some concern regarding calculations of pre-implantation loss in the two-generation study submitted by the applicant. We have identified that in the calculations the females stated as "Not littered" were not included.

1. The number of "Not littered" are as given in Table 2.

Table 2: Parturition performance: Not littered

Generation	Control	60 mg/kg bw/day	180 mg/kg bw/day	540 mg/kg bw/day

P	2	5	4	8
F1	0	0	4	5

2. "Not littered" females do **have corpora lutea** (CL), but **no implantation** (IM), still they are not included in the calculation of pre-implantation loss. As an example: The pre-implantation losses in the study report for P-generation are: **15.9 %** for the control and **18.6 %** for the 540 mg/kg bw/day group. If one includes the "Not littered" females in the calculations of pre-implantation loss the numbers are approximately: **23 %** for the control and **40 %** for the 540 mg/kg bw/day group.

   We did not find an explanation in the study report why "Not littered" females were not included in the calculation of pre-implantation loss as would have been expected.

RAC's response

The additional information was helpful and is considered critically in the RAC Opinion.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2014	Germany		MemberState	14

Comment received

Based on the presented data in the dossier we cannot follow the argumentation for the proposed classification Repr. 2, H361f. The observed increased oestrus cycle length in F1 females after treatment with 540 mg/kg bw/day (highest dose) is within the historical control range (4.3-4.5). Furthermore, in the highest dose group the body weight gain was reduced up to 30% in both sexes. Therefore, the increased oestrus cycle, the reduced fertility index and fecundity index may be due to reduced body weight gain. In this context, the effect on fertility seems to be minimal, but there are no information about historical control data for fertility index and fecundity index in this dossier. To decide about a possible classification the following data should be presented:
- individual data per animal of fertility index and bw gain
- historical control data of fertility index
- data relating to spermatogenesis (data on sperm production, i. e. number, motility, morphology)

Dossier Submitter's Response

Thank you for your comments.

We have uploaded the study report for the two generation reproduction toxicity study (Confidential 2008 /A6_8_2(3)) and the historical control data from 2012 (fertility and fecundity index, confidencial data) in the confidential folder on CIRCABC for your information (please refere "HISTORICAL DATA REPRO STUDY 2012" and "TWO GEN REPRO STUDY 2008").

Concerning the body weight gain, please see comment 13. The reduced body weight gain in exposed groups are less than 12% compared to the control group in females during gestation (Table 1).

RAC's response

Noted. The fertility classification proposal was initially unclear, but the information provided during the public consultation has been helpful. The key factor is the fertility index of the female rats in the 2-generation study.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2014	France		MemberState	15
Comment received				
Major Comments: Concerning ecotoxicity results, no acute toxicity data on fish are available. Taking into account the result of chronic studies, fish is considered as the most sensitive species with a very low NOEC (0.58 µg/L). Therefore, we wonder if 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 enough conservative. We are of the opinion that more data on acute toxicity to fish is needed confirm the acute M-factor.				
Dossier Submitter's Response				
Thank you for your comment. We think you have a good point in that the chronic studies show that fish are the most sensitive group, and that this gives rise to the question whether algae actually represent the most acutely sensitive trophic level and whether this uncertainty should be taken into account in the acute M-factor. Even though we cannot see that the practical consequences of the acute M-factor will be of notable significance for e.g. the classification of mixtures containing chlorophene, provided that the proposed chronic classification remains, we think this point should be raised in the further discussions. Please also see our response to comment no. 32 (under the subheading "Acute aquatic toxicity".				
RAC's response				
Noted. RAC's opinion is based on the information provided by the DS and any additional information provided during PC.				

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	16
Comment received				
The Netherlands agrees with the proposed classification of Acute Tox. 4 (H332) based on the LC50 (dust/mist) of 2.43 mg/L/4h which complies with inhalation (dust/mist) LC50 >1 but ≤ 5 mg/L for category 4 (Annex I:3.1.2 to 3.1.3.4).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Finland		MemberState	17
Comment received				
The Finnish CA agrees with the proposed classification of Chlorophene as Acute Tox. 4: H332 according to the Regulation (EC) No 1272/2008 (CLP).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	18

Comment received

The Netherlands agrees with the proposed classification of Skin Irrit. 2 (H315) based on an all 3 rabbits exhibiting oedema and erythema with mean values ≥ 2.3 to ≤ 4 which is the criteria for category 2 (Annex I:3.2.2.7 in CLP). However, please provide additional information on the studies to allow an evaluation whether the observed skin necrosis occurred through the epidermis and into the dermis or not. Was histopathology performed?

Dossier Submitter's Response

Thank you for your support. No histopathology was performed in the studies, and more specific information regarding the observed skin necrosis in the key study (Confidential, 2000 /A6_1_4(1)) was not given. There was only a short description of the effects observed in this study such as blanching, desquamation, necrotic appearing areas, eschar, scar-like tissue and exfoliation. The scar-like tissue was still present within the test site of all three animals at Day 21 of the observation period. Some of the effects were observed at day 7, 14 and 21 only, and these days have now been included in an extended version of Table 11 in the CLH-report:

Skin irritation (individual scores) of chlorophene (confidential, 2000 /A6_1_4(1)), extended table

Observation time	Rabbit no.					
	F05790		F05791		F05792	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
4 h	2b	4	2	4	2b	4
24 h	2b	4	2b	4	2b	4
48 h	3b	4	2b	4	2b	4
72 h	4n	4	4n	4	4n	4
96 h	4n	4	4n	4	4n	4
7 d	4x	3	4x	2	4e	4
14d	1d,s	0	0s	0	0s	0
21d	0s	0	0s	0	0s	0
Mean value 24 + 48 + 72 h	3.0	4.0	3.0	4.0	2.7	4.0
Reversibility	Yes	Yes	Yes	Yes	Yes	Yes
Average time for reversion	21 d	14 d	14 d	14 d	14 d	14 d

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

RAC's response

The additional information was helpful; please refer to the Opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Finland		MemberState	19

Comment received

The Finnish CA agrees with the proposed classification of Chlorophene as Skin Irrit. 2: H315 according to the Regulation (EC) No 1272/2008 (CLP).

Dossier Submitter's Response

Thank you for your support.

RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2014	France		MemberState	20
Comment received				
4.4.2.2 Comparison with the CLP criteria As we understand, the observation period was stopped before 21 days. Could you please specify which criteria for classification with eye damage is fulfilled because you mentioned all of them?				
Dossier Submitter's Response				
Thank you for your comment. Chlorophene produced ocular irritation characterised by diffuse opacity or translucency of the whole visible corneal surface, injection of the conjunctival blood vessels and eversion of the eyelids due to moderate chemosis. Positive irritation reactions were observed in all animals. Irritation responses of the treated eyes and other effects of treatment became more marked at each subsequent examination and culminated in a state of severe ocular irritation four days after treatment. At this time there was an ocular discharge, the conjuctivae had a beefy-read apperance, the eyelids were everted by moderate chemosis, the whole surface of the cornea was opalescent and the coulour of the iris indicated marked congestion of that tissue. In addition, areas of ulceration had exposed part of the cornea stroma of two rabbits. The study was terminated 72 h after treatment in light of the deteriorating condition of the treated eyes, especially the corneae. It was considered that significant resolution of the treatment effects was most improbable within the period of extended observation allowed by the OECD test method, and the study was terminated due to animal welfare reason. Evaluation criteria for classifying a substance for local effects on the eye are *severity* of eye damage and *reversibility*. The classification criteria for serious eye damage (Eye dam. Cat 1) was not met for chlorophene with regard to severity of the reported ocular lesions; one animal only had a mean score of ≥3 for corneal opacity and none a mean score of > 1,5 for iritis following grading at 24, 48 and 72 hours after installation of the test material. However, as the observed effects were not expected to reverse within an extended observation period, a classification with eye damage (Eye dam. Cat 1) was proposed for chlorophene.				
RAC's response				
The additional perspectives provided by the Dossier Submitter helped to clarify the proposal.				

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	21
Comment received				
The Netherlands agrees with Eye Dam. 1 based on the severity of the eye effects warranting termination of the study and because the effects are not expected to be reversible.				
Dossier Submitter's Response				

Thank you for your support.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	22
Comment received				
The Netherlands agrees with the proposed classification for Skin Sens. 1A (H317) given that in the Buehler's test, there were ≥ 60% respondents at > 0.2 to ≤ 20% topical induction (Table 3.4.3 in Regulation (EC) 1272/2008, CLP).				
Dossier Submitter's Response				
Thank you for your comment. According to the test guideline for the Buehler test (OECD 406) a pilot study should be performed to decide on the appropriate dose-levels for the main study. The concentration used for each induction exposure should be the highest to cause mild irritation, whereas the concentration used for challenge exposure should be the highest non-irritating dose. In the key study from 2001 (Confidential, 2001 / A_6_1_5), the result of the pilot study was that no dermal irritation was observed with 1 % chlorophene, very faint erythema reactions with 5 %, faint-moderate reactions with 10 %, and moderate-strong reactions with 25 %. In spite of these results, test concentrations of 10 % and 5 % were chosen for the induction and challenge phase respectively in the main study. As the test concentrations of chlorophene, as correctly pointed out by Finland and the applicant, were too high according to the requirements in the test guidelines both in the induction and challenge phase, the results of the study should be interpreted with care. The submitted data on sensitization was re- evaluated during the public consultation, and a revised classification proposal was made based on all available information in a weight of evidence assessment, including also new information provided by the applicant in the public consultation. Hence, our new proposal for classification of chlorophene is Skin Sens 1. This proposal is in accordance with the CLP guidelines (*If the classification for subcategory 1A may not be excluded, the substance should be classified as a Category 1 skin sensitizer.).* Further comments on this endpoint could also be found in comment number 24.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Finland		MemberState	23
Comment received				
The Finnish CA is of the view that the data present in the CLH report is not sufficient for classification of Chlorophene as Skin Sens. 1A; H317 according to Regulation (EC) No 1272/2008 (CLP). The key study Buehler test OECD 406 on which the classification proposal is based on is not well enough described in the CLH report. Although the study is marked as confidential, we think that all relevant data should be described thoroughly in the CLH report to allow its evaluation. Because Chlorophene has skin irritating properties, a pilot study should have been conducted to determine the appropriate test concentrations for induction and challenge phases of Buehler test. This information is missing from the report. In the key				

study 10 % w/v mixture caused moderate to strong erythema reactions during induction phase and also subcutaneous haemorrhaging, blanching, and necrotic appearing areas were observed in the test animals. According to the OECD test guideline 406 the concentration of the test substance used for the induction exposure should be the highest to cause mild irritation. It seems that the test concentration used for the induction phase was too high and this causes some uncertainties about the reliability of the results and validity of the study. These results would meet the criteria as Skin Sens. 1A (≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose) but they should be re-evaluated.
In another Buehler test OECD 406, which is also marked as confidential, 45 % of animals had skin reactions when tested with 50 % concentration for both induction and challenge phases. The validity of the study cannot be evaluated as the detailed description of the study design and results are missing. Based on the reported results, the criteria as Skin Sens. 1B (≥ 15 % responding at > 20 % topical induction dose) is fulfilled.
Because there are uncertainties about the validity of the two Buehler tests, the Finnish CA does not support the classification Skin Sens. 1A; H317.

Dossier Submitter's Response

Thank you for your comment.
We agree with the Finnish CA that the concentration of chlorophene used in the induction phase is too high and that the classification into category 1A should be reconsidered.

In the pilot study to a Buehler test given in the CLH-report (*Confidential, 2002. This study report is uploaded as "BUEHLER TEST 2002 NON KEY CLH" in the confidential folder on CIRCABC. Although the study is included in the original dossier for chlorophene, the study summay is not part of the confidential Annex 1 to the CLH report as this is a non-key study)* no irritation was observed using up to 50% chlorophene. The authors, therefore, chose to use the highest tested concentration both in the induction and challenge phase. We are of the opinion that the dose levels are not set properly and thus the study should not be used to sub categorise chlorophene into either category A or B.

Based on all available information, including the new information provided by the applicant in the public consultation, we would propose to classify chlorophene as Skin Sens 1.

Further comments on this endpoint could also be found in comment number 24.

RAC's response

Noted; see Opinion for detailed assessment.

Date	Country	Organisation	Type of Organisation	Comment number
25.09.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	24

Comment received

4.5 Skin Sensitization

Lanxess does not agree with the proposed classification with Skin Sens Cat 1A (H317). Rather, Lanxess proposes classification with Skin Sens Cat 1B (H317) for the following reasons:

A Buehler Test (confidential 2001, A6_1_5, Key Study) was taken as basis for classification with Cat 1A by the Norwegian Environmental Agency. The test result is reported as "faint to moderate erythema in 19/20 animals with sporadicly appearing subcutaneous hemorrhaging, desquamation and fissuring" after challenge. Two of the 10 control animals

treated with chlorophene at challenge also showed skin erythema, thus, a final skin sensitizing frequency of 75% appeared in this study. However, this study has major deficiencies that render - not the positive result per se, but - the height of the result questionable. The test concentrations chosen for induction and for challenge were too high and thus, not appropriate. In the dose range finding test of the Buehler Test (confidential 2001, A6_1_5) 10% chlorophene in propylene glycol led to faint (score 1, 2/4 animals) and moderate (score 2, 1/4 animals) erythema. Although showing such distinct irritating effects, as induction concentration for the main experiment 10% was chosen which induced moderate (grade 2, 2/20 animals) to strong irritation with necrotic appearing areas (grade 3, 18/20 animals) after the second induction treatment. As a consequence, the treatment area had to be changed for the third induction. Treatment on this new skin area led to strong erythema (grade 1 to 3 in all animals) with subcutaneous hemorrhaging and necrotic appearing areas in 8/20 animals, possibly the consequence of an arising excited skin syndrome, also called angry skin syndrome (Andersen and Maibach, 1980*). According to OECD TG 406 the concentration used for each induction exposure should be the highest to cause mild irritation and all induction treatments should be carried out on the same test area. Thus, clear deviations from guideline become obvious which may have influenced the outcome of the test. For challenge a concentration of 5% in propylene glycol was used, although in the dose finding study this concentration led to faint erythema at one test side. According to OECD 406 the concentration for challenge should be the highest non-irritating dose.
In conclusion the deviations shown here do not allow taking this study as solely basis for classification of the skin sensitizing potential of chlorophene. Rather, a weight of evidence assessment should be conducted which includes all relevant information:

• The Buehler test (confidential 2001, A6_1_5, Key Study), described in detail above, showed a frequency of 75% Guinea pigs with skin effects at the moderately to strong irritating concentration of 10% for induction and the slightly irritating concentration of 5% for challenge in propylene glycol. As explained above, these concentrations were much too high and are not conform to Guideline requirements. The data suggest that the development of an excited or angry skin syndrome (Andersen and Maibach, 1980), a syndrome of nonspecific hypersensitivity related to the experimental conditions, may have potentiated the effects.

• In an Open Epicutan Test (Klecak Test, confidential 1986) 8 Guinea pigs per group were treated 20 times during 4 weeks with 1, 3, or 10% chlorophene in propylene glycol, the same vehicle and similar concentrations as used in the Buehler test (2001, A6_1_5). After the first 5 days of treatment the concentration was reduced in the 10% group to 3% and the treatment area was changed due to strong and cumulative skin effects (erythema, oedema and encrustation in all animals). Challenge was started 2 weeks after induction with 0.3%, 1%, and 3% chlorophene. In this test no skin sensitizing effects were observed.

• In a second Buehler Test performed (confidential, 2005*) on 10 Guinea pigs per group (number not Guideline conform) chlorophene was applied in a concentration of 0.5% in ethanol/water (80/20) for induction. In the dose finding study this concentration led to very faint desquamation in 1 of 4 animals. For challenge the next lower concentration of 0.25% was used (vehicle: acetone). The concentration chosen for induction might have been little too low, 1% that led to slight effects in all animals would perhaps have been more appropriate. After challenge very faint erythema was seen (score 0.5) in 4 of 10 induced animals and 2 of 10 control animals. Overall, the frequency of very mild response in the test group compared to the control group is 20% and thus, falls into Cat 1B for classification.

• In a third Buehler Test (confidential, 2002) according to OECD 406 with 20 animals/test

group 50% chlorophene in polyethylene glycol 400 was used for induction and challenge. Apparently, chlorophene exerts much lower irritating properties in this vehicle than in the vehicles used in the tests described above. A dose finding study with different concentrations up to 50% did not show skin irritation. Slight irritation became obvious after the third induction treatment in 7 of 20 animals. At challenge, slight to moderate skin effects were induced in 45% of the animals (9 of 20). No effects were seen in the control animals. Thus, this test result falls into Cat 1B for classification.

• Buehler Tests with chlorophene, tested as component in two disinfectant formulations at low concentrations (1% and about 0.2% chlorophene, respectively, for challenge) showed negative results (confidential, 1998a* and 1998b*). Although these tests are of minor relevance for chlorophene assessment they nevertheless confirm the view that chlorophene is not a strong skin sensitizer and that classification of chlorophene with Skin Sens 1A is not appropriate.

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

• In humans, although chlorophene is widely used as disinfectant in professional and private settings, it seems to having only low skin sensitizing potency. Reports are available in which 1 of 221 patients reacted positive to 25% chlorophene in water (Dohn, 1980*) and 7 of 371 humans with a suspected contact dermatitis towards disinfectants reacted positive to pure chlorophene (Rothe et al, 1993*). In one case study a person reacted positive to 1% chlorophene, whereas of the 50 control subjects 47 did not show any reaction to chlorophene and 3 showed mild irritant reactions only (Sonnex and Rycroft, 1986*). In an early publication of Kahn et al. (1970*) 3 of 13 persons reacted positive to chlorophene as well as to two other phenolic constituents tested, indicating that cross-hyperreactivity can occur.
In conclusion, taking all available information into account, chlorophene should be considered as skin sensitizer with a mild to moderate potency, thus, classification with Skin Sens Cat 1B seems appropriate.

* data have been submitted to the RMS Norway by Lanxess recently, i.e.
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, 430-434
Confidential, 2005. Chlorophen: Dermal sensitization study in Guinea pigs – closed patch technique.
Confidential, 1998a. Examination of Preventol CD 590 in a skin sensitization test in Guinea pigs.
Confidential, 1998b. Dermal Sensitization Test (Buehler method) with Phenocide 256.
Schlede E et al., 2003. Chemical substances and contact allergy – 244 substances ranked according to allergenic potency. Toxicology 193, 219-259
Dohn W, 1980. Dermatological patients not employed in handicraft or factories. Contact Dermatitis 6, 148-150
Rothe A et al., 1993. Contact dermatitis caused by formaldehyde-free disinfectants. Hygiene Medizin 18, 167-175
Sonnex TS and Rycroft RJ, 1986. Allergic contact dermatitis from orthobenzyl parachlorophenol in a drinking glass cleaner. Contact Dermatitis 14, 247-248
Kahn G,1970. Depigmentation caused by phenolic detergent germicides. Arch Dermatol 192, 177-187

ECHA note: The following attachment was provided [Attachment 2]

Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section.
(*The content of this attachment, except for the References on the last two pages, is already available in this comments table*)

Dossier Submitter's Response

Thank you for your comments. Please find our response below.

Comments on the key study in the CLH-report (Buehler test, Confidential, 2001/ A6_1_5):
We agree that the concentrations of chlorophene used in the induction and challenge phase is too high. See also response to comment number 22.

Comments on the Klecak Test in the CLH-report (Confidential, 1986):
In this study strong cumulative skin effects like erythema, edema and encrustation was observed, but no skin sensitization. This study, however, is not a guideline study and has of that reason not been regarded as that relevant in the weight of evidence approach for chlorophene.

Comments on the Buehler Test submitted in the public consultation by the applicant (Confidential, 2005. This confidential study report is uploaded as "BUEHLER TEST 2005" in the confidential folder on CIRCABC):
We agree that the concentrations used in the study provided might be too low. Furthermore, as pointed out by the applicant, only 10 animals were used in the test groups instead of the recommended number in the guideline. In our opinion, this study give support to classify chlorophene as a skin sensitizer, but the data cannot be used for further sub-categoriseing into category A or B.

Comments on the other Buehler Test given in the CLH-report (Confidential, 2002. Uploaded as "BUEHLER TEST 2002 NON KEY CLH" in the confidential folder on CIRCABC):
In the pilot study of this Buehler test, no irritation was observed using up to 50% chlorophene. The authors, therefore, chose to use the highest tested dose both in the induction and challenge phase. We are of the opinion that the dose levels were not set properly and thus the study cannot be used to sub categorise chlorophene into either category A or B.

Comments on "Buehler tests with chlorophene, tested as components in two disinfectant formulations" submitted by the applicant in the public consultation:
As already highlighted by the applicant these studies were conducted with two different disinfectant formulations. Neither the concentration of chlorophene, nor the other ingredients in the formulation were given in the report. The results are of minor relevance for the assessment of chlorophene since formulations were tested and not the active substance per se and were not taken into account.

Comments on "Schlede et al., 2003":
This publication ranks chemical substances into three categories based on the following definitions
Category A – significant contact allergen because of:

1. Proven strong contact allergenic effect in humans after short and/or almost negligible exposure taking into account existing animal data
2. Frequently proven contact allergenic effects in humans

Category B – solid-based indication for contact allergenic effects because of:

1. Less frequently proven contact allergenic effect in humans taking into account existing positive animal data
2. The capacity of substances to induce cross-reactions in humans without being a significant allergen itself

Category C – insignificant contact allergen or questionable contact allergenic effect because of:

1. Rarely proven contact allergenic in humans
2. Doubtful effect in humans; no or non-appropriate animal data
3. No data on humans but positive animal data

According to this publication, there are human clinical data and negative human experimental data on chlorophene. In addition, there are both positive and negative animal studies.
We do not know which studies they used for the categorization in this publication, i.e. whether the studies provided by the applicant were included in their assessment. Since these studies give evidence for skin sensitizing properties of chlorophene, they could have influenced the categorization made by Schlede et al.

Comment on human data:
There is some evidence in humans that chlorophene is a sensitizer, although it might be difficult to use these data for classification. Even though the animal studies provided by the applicant have some limitations, there is enough evidence to classify chlorophene as a skin sensitizer category 1 based on these tests.

Final conclusion:
Both animal studies and results from humans on chlorophene give evidence that chlorophene is a skin sensitizer. However, due to deficiencies in the animal studies (including choice of test concentration) and few human data all with limitations, we are of the opinion that neither animal nor human studies can be used for further sub categorisation into category 1A or 1B. Hence, we propose to classify chlorophene as Skin Sens 1.

RAC's response
The additional information was helpful in clarifying the skin sensitisation potential of chlorophene.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	25
Comment received				
The Netherlands requests additional justification on the kidney effects in the dermal rabbit studies that would justify classification as STOT RE 1. The only effect increased compared to the controls seem to be tubular calcinosis (score 2) in 3 rabbits at 40 mg/kg bw/day compared to none in the controls. Please provide information what score 2 means (marginal increase?). Please justify why this effect at score 2 is considered significant organ damage.				
Dossier Submitter's Response				
Thank you for your comment. Please see our response to comment number 28.				
RAC's response				
Noted. RAC agrees on the importance of the tubular calcinosis in rabbits.				

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Finland		MemberState	26
Comment received				
We agree that the data presented in the CLH report warrants classification for STOT RE due to kidney effects. However, we are not certain whether classification for STOT RE 1, based on 3-week dermal study in rabbit, is warranted. The data set presented suggests that rabbit is the most sensitive species for chlorohene kidney toxicity, and the 3-week rabbit study gives the most severe classification after adjustment of the LOAEL for study duration. Yet, adjustment of LOAEL should be done with caution and longer studies should generally have preference in judgement. We think that to enable proper weight of evidence assessment and to justify STOT RE 1 classification, the selected studies, especially the rabbit study, should be reported more accurately in the dossier.				
Dossier Submitter's Response				
Thank you for your coment. Please see our response to comment number 28.				
RAC's response				
RAC has considered all available studies.				

Date	Country	Organisation	Type of Organisation	Comment number
25.09.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	27
Comment received				
4.6 Repeated Dose Toxicity Lanxess does not agree with the proposed classification with STOT RE 1 (H372). Rather, Lanxess proposes a classification with STOT RE 2 (H373) for the following reasons: 1. As already noted by the Norwegian Environmental Agency, summarized in Chapter 4.6.2. 'Comparison with the CLP criteria' (page 38 of the CLP Dossier), the following repeated dose toxicity and key studies performed with chlorophene warrant a classification with STOT RE 2: a) the oral 2-year study with a LO(A)EL of 30 mg/kg bw in F344 rats (NTP, 1994, A6_5+6_7; 160 rats/dose) b) the 2-generation study in Wistar rats equivalent to a 90-day repeated-dose study with a LO(A)EL of 60 mg/kg bw (confidential, 2008, A6_8_2(3); 30 rats/dose/generation) c) the 90-day study in Beagle dogs (confidential, 1973b A6_4_1(2); 8 dogs/dose), although Lanxess does not agree with the LOAEL of 30 mg/kg bw/day determined by the Norwegian Environmental Agency This LOAEL was based on a statistically significant increase in relative kidney weight in male dogs, the absolute kidney weight of males was not altered. However, in female dogs the absolute kidney weight was reduced at this dose and the relative weight was not altered. Thus, Lanxess does not interpret these contradictory findings as adverse and determines the LOAEL at the next higher dose of 100 mg/kg bw/day. For classification this result is a STOT RE 2/no classification borderline. 2. No classification at all is warranted for the key study of NTP with subchronic exposure of rats (Birnbaum et al. 1986, NTP, 1994, A6_4_1(1); 20 animals/dose). Also the subchronic /chronic studies in mice (Birnbaum et al. 1986, NTP, 1994, A6_4_1(1) and A6_7 (1); 20 and 140 animals/dose) do not trigger classification. 3. The only study which is under discussion as basis for STOT RE 1 classification is a 21 day dermal toxicity study on rabbits, reported in 1985 (confidential, 1985, A6_3_2(2)). In this study 5 New Zealand white rabbits per sex were treated 6h/d, 5d/wk by topical application				

of chlorophene in doses of 0, 10, 40, and 160 mg/kg bw in Lutrol. One male of the control group died on day 5. Local irritating effects were seen at the mid-dose of 40 mg/kg bw starting on the second day of application. At day 5 all animals of this dose group showed skin reddening (grade + and ++) and oedema prior to and after treatment. At termination all animals of this group showed skin lesions, histopathologically determined as e.g. acanthosis, keratosis, oedema, or hyperplasia of sebaceous glands. The animals of the 160 mg/kg bw group showed such strong skin lesions that the application area was changed several times during the treatment period. It can be considered that the animals of the mid and high dose group have suffered from the treatment procedure related to the irritation potential of the substance as applied.
With regard to systemic effects Lanxess does not agree with the proposed LOAEL of 40 mg/kg bw of the Norwegian Environmental Agency which is based on histopathological findings in the kidney. Nephrotoxic effects were also recorded for 4 of the 9 control animals (1 control male died on day 5), with cellular infiltration (grade 1 to 3) and tubular proliferation (grade 2). In the 40 mg/kg bw group nephrotoxic effects occurred in 7 of 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, which is not an uncommon finding in the rabbit kidney. Neither urinalysis parameters nor serum creatinine and urea levels were changed in treated rabbits at any dose, proving clear evidence that no functional changes have been induced and no kidney damage had occurred.
Thus, we see the LOAEL for systemic effects at 160 mg/kg bw, since at this dose the frequency and severity of nephrotoxic effects is increased compared to control which indicates responses that can be interpreted to be adverse (even if the directors of the respective 21-day dermal study in rabbits (confidential, 1985, A6_3_2(2)) stated that “The clinical-chemical, gravimetric, pathological-anatomical and histopathological investigations showed that rabbits in the groups up to and including 160 mg/kg had no kidney damage.”). In addition, the liver is affected (liver weight and alkaline phosphatase activity reduced, reduced storage of presumably glycogen in the liver) at this dose. Consequently, with adverse effects starting at 160 mg/kg bw/day also this study would lead to classification with STOT RE 2.

Classification should be based on reliable studies with relevant laboratory animal species and all available information should be taken into account. Reliable, guideline compliant studies on rats, mice and dogs with treatment durations of up to 2 years are available. We don’t agree with the decision of the Norwegian Environmental Agency to base their classification proposal solely on an older study on 5 rabbits/sex/dose with a 15 day dermal treatment regime (confidential, 1985, A6_3_2(2)); see description above), which led to strong skin irritation and, thus, to stress and distress by the treatment procedure itself. This study is as such no ‘appropriate study’ as requested for classification in CLP Annex 1, 2.9.2.7.3.
Additionally, there is no indication that chlorophene produces systemic toxicity in humans although used widely as biocide for professional and private use. Medical surveillance of manufacturing plant personnel involved in chlorophene production revealed no health complaints associated with potential exposure to chlorophene (A6_12_1; Confidential, 2007) (Confidential, 2013*).
In conclusion, taking all information into account, chlorophene should be classified with STOT RE 2 (H373).

* data have been submitted to the RMS Norway by Lanxess recently, i.e.
Confidential, 2013. Medical statement – 2-benzyl-4-chlorophenol (BP)

ECHA note: The following attachment was provided [Attachment 2]

Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section.
(*The content of this attachment, except for the References on the last two pages, is already available in this comments table*)

Dossier Submitter's Response
Thank you for your comment. Please see our response to comment number 28.
RAC's response
Noted. Please refer to the Opinion for detailed assessment of this endpoint.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2014	Germany		MemberState	28

Comment received

Repeated dose toxicity:
Based on partly poor data description we cannot follow the argumentation for the proposed classification STOT RE 1, H372.
We agree with the dossier submitter that for chlorophene the kidneys seem to be the target organ. However, it is questionable whether the effective dose is between the classification limits for STOT RE.

Concerning the studies which are discussed for classification we have the following comments:
Rabbit studies:
Data description is poor for the dermal rabbit study on which the proposed classification is based. In our opinion, the kidney lesions observed in a dermal study in rabbit [confidential, 1985 / A6_3_2(2)] are not sufficient for classification as STOT RE 1. Also, a classification as STOT RE 2 seems to be questionable. In the mid-dose group (40 mg/kg bw/day), tubular calcinosis is the only histopathological change in the kidney with increased incidence and severity compared to the control group. The discussion why this constitutes a severe effect is missing. Which clinical consequence has this histopathological change? Furthermore, in the second 3-week dermal study in rabbit [confidential, 1985 / A6_3_2(1)], quantitative data for kidney lesions are missing.
To decide about a possible classification, data on histopathological data and data on clinical-chemistry should be included and presented.
Rat studies:
In relation to the oral 3-year study in F344 rats, chronic progressive nepthropathy is a common finding in F344 rats. After 2 years there is a minimal increase in severity compared to the control group, however this is beyond the classification limit of 25 mg/kg bw/day for STOT RE 2.
The evaluation of the kidney lesions observed in a 2-generation study in Wistar rats is based on the same argument. In the mid-dose (180 mg/kg bw/day) kidney lesions were observed in male rats, but this is beyond the classification limit of 100 mg/kg bw/day for STOT RE 2. Regarding systemic effects the 2-generation study is equivalent to a 90-day repeated-dose study.
Dog study:
There are no quantitative data for the 90-day study in Beagle dogs. An increase in relative kidney weight is not sufficient for classification in STOT RE. There are no data on histopathological findings. In relation to the observed hyposthenuria there are no quantitative data on incidence and severity. In our opinion data on body weight gain and food consumption are also important for the evaluation of the hyposthenuria.
To decide about a possible classification, the dossier should include quantitative data on

incidence and severity of the hyposthenuria, histopathological data and clinical-chemical data as well as individual data per animal of bw gain and food and water consumption.

Dossier Submitter's Response

The reasons for proposing a classification as STOT-RE was based on adverse findings in rats, and supporting evidence in dogs and rabbits. As described in chapter 4.6.2 in the CLH-report, Fischer 344 rats in the oral 2-year study had a LO(A)EL of 30 mg/kg bw/day based on severity of nephropathy that was significantly increased at 30 mg/kg in males at 65 and 104 weeks. In the 2-generation study in Wistar rats, equivalent to a 90-day repeated-dose study, treatment-related kidney effects (nephropathy, dilated tubules, basophilic tubules and lymphocytic infiltration) were observed in P and F1 males at ≥ 60 mg/kg leading to a LO(A)EL at the lowest dose tested (60 mg/kg bw/day). The kidney effects in these two rat studies are supported by the findings in the 90-day Beagle dog study with a LO(A)EL of 30 mg/kg bw/day. Based on the LO(A)EL in the three studies mentioned above a classification as STOT-RE 2 would be appropriate.

Based on the effects observed in the 3-week dermal study in rabbits, with effects in the target organ, the kidney, and showing a dose-dependent increase in the sum of all histopathological changes and a LO(A)EL of 40 mg/kg bw/day led to the proposal of STOT-RE1. We have now conducted a comprehensive re-evaluation of the rabbit study, taking into account the comments received.

Further information regarding the Beagle dog study (90-day oral by capsule) and the rabbit study (3-week dermal) is included below.

Beagle dog study [Confidential 1973b / A6_4_1(2)]):
This study is a pre-guidance study with limitations. Results from the Beagle dog study is presented (Figure A, B and C). Weight loss was seen in the highest dose group of 200 mg/kg bw/day, and at 100 mg/kg bw/day there was a significantly lower weight gain in both females and males. Relative weights of kidneys were significantly increased in a dose-dependent manner in male dogs. The means (Figure B) for all dose groups were significantly different from the controls, which could warrant a discussion for an even lower LO(A)EL. In female dogs the relative kidney weights were significantly increased at 100 mg/kg bw/day. The urine specific weights (Figure C) would normally be considered together with clinical examination and clinical chemistry values. No clinical observations were reported to support that the dogs were dehydrated. Urine volume was not reported. The clinical chemistry values were normal. Water consumption was not reported. Other results from urine analysis were normal. The observed low specific weights for urine may originate in polydipsia caused by symptoms from other organs. No observations were reported that support this possibiliy.

Figure A) Raw data Dog study

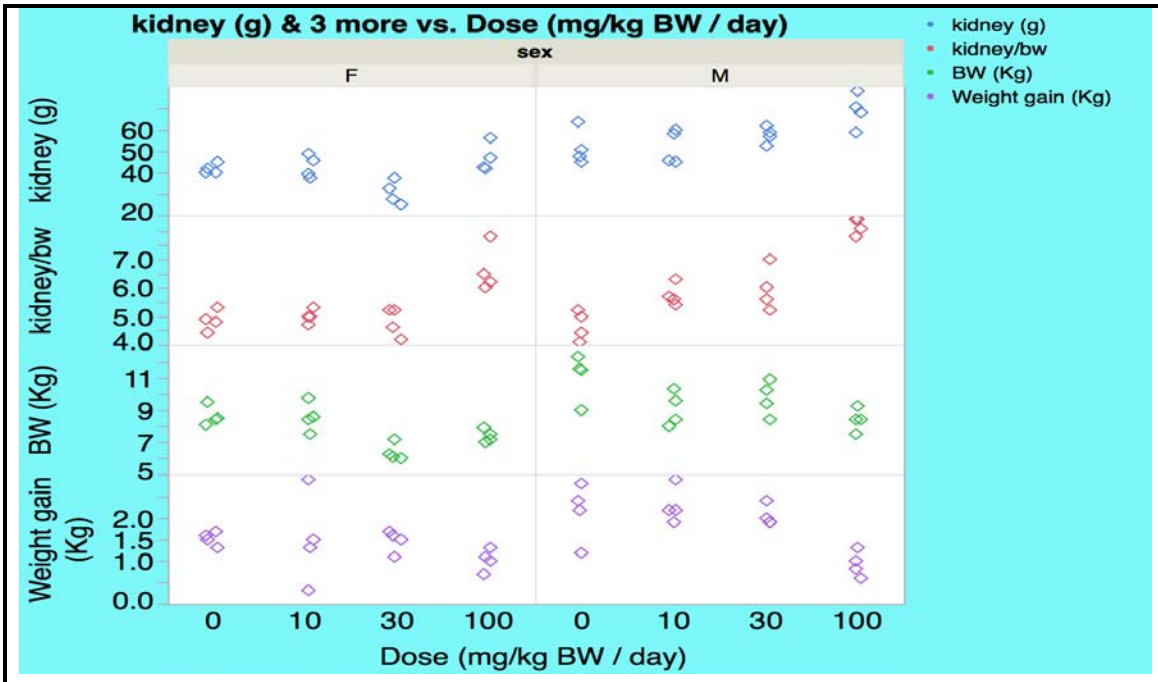
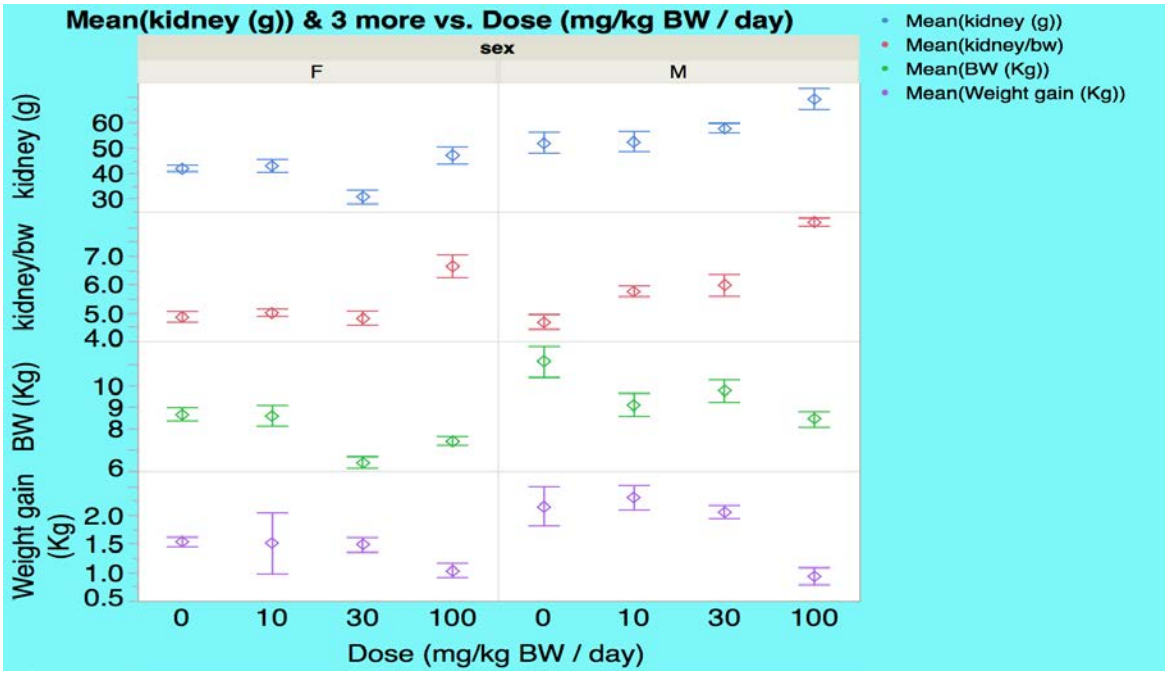
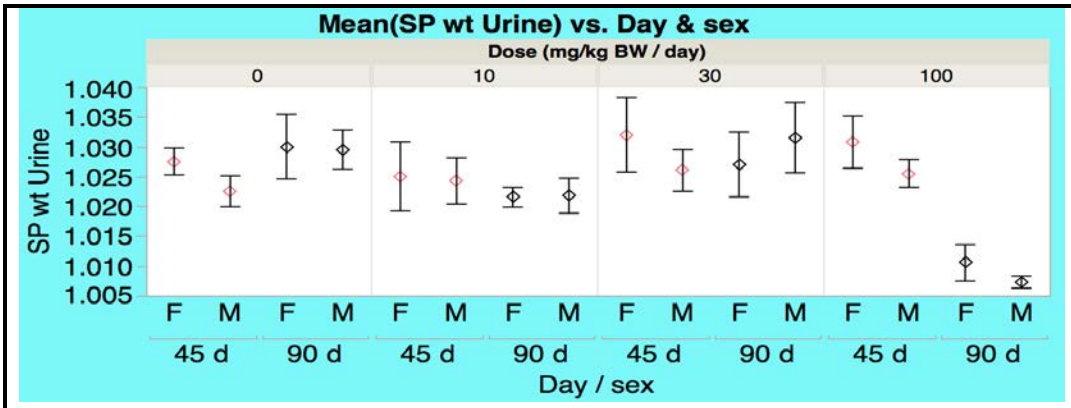


Figure B) Means +/- SE for the data presented in A) Dog study



Each error bar is constructed using 1 standard error from the mean.

Figure C) Urine specific weights Dog study



Each error bar is constructed using 1 standard error from the mean.

Rabbit study [Confidential 1985 / A6_3_2(2)]:
The results from a 3-week dermal study in rabbits led to the proposal of STOT-RE 1. This was based on a dose-response relationship when all histopathological effects were considered as a whole.
Some more details on the histopathological results may be in place: Calcinosis is a common finding in rabbits due to the peculiar calcium metabolism in this species where the kidneys play a very central role in regulating the plasma calcium level. The calcinosis should be regarded as a non-reversible condition.
The results in this study suggest a dose-response effect, with increased tubular calcinosis in the 40 and 160 mg/kg bw/day-groups. The tubular proliferation and mononuclear infiltration observed is a common finding in infections with *Encephalitozoon cuniculi* (as stated in the report). The grading of all lesions is from 1 to 5, where a score of 2 is "weak to medium" degree of severity, thus the observed lesions are not severe in any dose group. Clinical chemical and urinalysis values show no difference between the groups. Table 18 reveals that monocellular infiltration (common for *E. cuniculi*) is most prominent in control animals, while calcification is most prominent in the mid-dose group – it is lower in the high dose grop, and protein in lumen is only present in the high dose group. If all effects are combined we see a dose-response in this table. An infection would be regarded of no importance as long as the dose groups are compared with relevant concurrent controls. However, subclinical parasitic infections may lead to spontaneous pathological findings, and in a small study like this (N=29), correlations may not be of biological significance. Our re-evaluation of this study indicate that these results may not be sufficient to support a STOT-RE 1 classification. This should be further discussed in RAC.

RAC's response
Noted. Please see the RAC Opinion for a detailed assessment of this endpoint.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
20.10.2014	France		MemberState	29
Comment received				
Minor comments: - Considering the aerobic biodegradation study of chlorophene in soil (Nitsche, 2011), we are of the opinion that the study could not be considered as acceptable because only 1 soil was tested and there are several lack of relevant information such as degradation products, bound residues, mineralisation.				
Dossier Submitter's Response				
Thank you for your comment. We agree that there are several weak points in the aerobic				

degradation study in soil and therefore the reliability was adjusted from 1 to 2. However, we do not think the study is important for the conclusion that chlorophene is not rapidly biodegradable (see our response to comment no. 30 below, under the subheading "Biodegradation") and it should be considered as additional information only.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
16.10.2014	Netherlands		MemberState	30

Comment received

NL agrees with the conclusion that the substance cannot be considered readily biodegradable. However, it does not consider the results of the ready biodegradability tests ambiguous. Both tests showed that the substance is not ready biodegradable under the prevailing test conditions. In the CO2 evolution test (OECD 301B) the pass level was not reached in the 10-day window, while in the manometric respirometry test (OECD 301F) degradation was low (9% after 28 days). The concentration of the test substance (100 mg/L) in the latter test is considered acceptable, as it is below maximum solubility (0.117 g/L at 20°C), is in accordance with the test guideline (100 mg/L), and toxicity to inoculum was not shown, i.e. reference compound was degraded in the toxicity control, even though the EC50 for microorganisms (59.6 mg/L) was exceeded.

We cannot support the conclusion on bioaccumulation as relevant study details are missing. The measured lipid corrected BCF values (1130, 1401) are in the same range as the QSAR estimated BCF value (858.5). However, to conclude that the substance does not bioaccumulate the dossier submitter should demonstrate that steady state was reached in the short uptake phase of 8 days (which is usually 28 days) and report the LOQ and measured test concentrations so it can be determined if the water concentrations remained ±20% of the mean measured values during uptake phase. Furthermore, relevant study details such as the applied method (i.e. flow-through or semi-static), flow rates, mortality, and number of fish sampled per sampling point, fish-to-water loading rate and information concerning transformation products (measured/detected?) are to be included in the study summary.

Dossier Submitter's Response

Thank you for your comments.

Biodegradation

We agree that the wording in the report might be misleading and that based on the degradation of the reference substance, chlorophene might not have inhibited the inoculum in the manometric respirometry test. Regarding our description of the two tests as giving ambiguous results, this originates from the guidance documents for the risk assessment of biocidal active substances. Here, substances degraded > 60 % during the ready biodegradation tests which do and do not fulfil the 10 day window criterion are put in different categories when it comes to assigning degradation rates in sewage treatment plants. However, we acknowledge that for classification purposes, in order to categorise a substance as "rapidly degradable", the 60 % degradation level must be reached within the 10 day window. In this respect and for the purpose of the CLH report, we agree with you that the conclusions from the two tests on ready biodegradation are not ambiguous. The conclusion that chlorophene is not rapidly degradable thus seems clear from our side.

Bioaccumulation

The conclusion on bioaccumulation is not relevant for the classification of chlorophene, since

the chronic toxicity nevertheless warrants a classification of Aquatic Chronic 1. That being said, we acknowledge that more details should have been included in the CLH report itself in order to back up our conclusion on the issue, even though relevant study details are available in Document III-A7.4.3.3.1 (part of the confidential Annex 2 to the CLH report). We have listed further study details which are not/inadequately described in the CLH report:

Test setup
- flow-through conditions, water renewal rate of approx. 6 times per day, photoperiod of 16 h light and 8 h dark.
- Fish were kept in glass aquaria containing 40 L. Fish-to water loading rate: 0.1-1 g fish (wet weight) / L.
- Temperature: measured daily, ranged from 20 to 25 °C.
- pH: measured daily, ranged from 7.7 to 8.0.
- Test media was gently aerated via narrow glass tubes. Oxygen saturation: measured daily, ≥ 95 % throughout the test. Holding and dilution water: synthetic fresh water prepared according to ISO 7346-1 (1996).
- Number of fish in control and treatment groups of 3 and 15 µg chlorophene / L: 35, 37 and 47 animals, respectively.

Mortality
- Recorded daily, dead fish were discarded. During the study, one fish died (15 µg/L treatment group). All other individuals (controls and treatment groups) exhibited normal swimming action.

Sampling and analytical monitoring of chlorophene in test medium and fish
- Analytical monitoring (HPLC) in water and fish on day 1, 4, 6 and 8 of the uptake phase, and 6 hours after initiation of the depuration phase. Additional measurements (HPLC) of fish on day 9, 11 and 13 (i.e. day 1, 3 and 5 of the depuration phase).
- Sampling and pooling: At each fish sampling time, two control fish and four fish from each treatment group were sampled. For each analysis, two fish were pooled together.
- LOQ for chlorophene in water and fish: 0.7 µg/L and 0.09 µg/g fish, respectively.
- Measured chlorophene concentrations in water: Uptake phase: from 2 to 4 µg/L in the 3 µg/L treatment group (mean: 3.38 µg/L), from 15 to 18 µg/L in the 15 µg/L treatment group (mean: 16.88 µg/L). Depuration phase: No chlorophene detected at sampling 6 hours after initiation. No chlorophene detected in control water.
- Measured chlorophene concentrations in fish during uptake phase (corrected for 70 % recovery):

Treatment group	Day and mean chlorophene concentration in µg/g fish
3 µg/L	1: 0.329, 4: 0.387, 6: 0.333, 8: 0.395 (tot. mean*: 0.361 µg/g)
15 µg/L	1: 1.919, 4: 1.433, 6: 2.046, 8: 2.038 (tot. mean: 1.859 µg/g)

*) Total mean values are used for calculation of lipid-normalised BCF, see below.

Depuration phase, 6 hours after initiation: no chlorophene detected in fish in the 3 µg/L treatment group, 0.141 µg/g detected in the 15 µg/L treatment group. Depuration phase, 24 hours after initiation (day 9): no chlorophene detected in any fish. No chlorophene detected in control fish at any time.

Metabolites / transformation products
- Metabolites / transformation products were not measured.

Lipid content and lipid normalisation
- The lipid content of fish from the stock population (representative for both control and

treated fish) was measured at test start. At the end of the test, i.e. end of the depuration phase, the lipid content was measured in control fish and fish exposed to 15 µg/L. At test start, the mean lipid content of the stock population fish was 6.8 %. At test end, the mean lipid content of the control and treated fish was 8.5 % and 12.7 %, respectively. The overall mean lipid content of the control and treated fish was 7.65 % and 9.75 %, respectively. The increase is higher than recommended in the guideline. This issue could be addressed during the PBT discussion on the substance.

- Lipid normalisation: Unfortunately, there has been some confusion regarding terminology. The values of 1401 and 1130 presented in the report are not the lipid-normalised BCF values, they are the BCF values in the lipid fraction of the fish. The following recalculation gives the lipid-normalised BCF values for chlorophene:

Lipid-normalised concentration $C_{f,L}$ in fish [eqn. A5.29 in OECD 305]:

$C_{f,L} = (0.05 / L) \cdot C_f$

$L = 0.0975$ is used for both the 3 and 15 µg/L treatment group

$C_f = 0.361$ and 1.859 mg/kg for the 3 and 15 µg/L treatment groups, respectively (cf. analytical monitoring comment above)

$C_{f,L}$ for the 3 µg/L treatment group = 0.185 mg/kg
$C_{f,L}$ for the 15 µg/L treatment group = 0.953 mg/kg

Lipid-normalised BCF = $C_{f,L} / C_w$

$C_w = 0.00338$ and 0.01688 mg/L for the 3 and 15 µg/L treatment groups, respectively (cf. analytical monitoring comment above)

Lipid-normalised BCF for the 3 µg/L treatment group = **55 L/kg***
Lipid-normalised BCF for the 15 µg/L treatment group = **56 L/kg**

*) Lipid content was measured only in the control and 15 µg/L treatment group. If a lipid fraction of 0.0765 (from the control group) had been used for the 3 µg/L treatment group, the lipid-normalised BCF would be 70 L/kg.

<u>Size/weight of fish</u>

- In the study report, it is stated that fish were weighed (fresh weight) and metered every time fish samples were taken. However, only the weight and length of the fish in the stock population is reported. The individual fish weight ranged from 365.5 mg to 590.4 mg, and the length ranged from 3.6 to 4.2 cm.

<u>Deviations from length of uptake and depuration phase, comment on steady-state</u>

- As stated in the report, pre-tests indicated that the bioconcentration of the test item might be fast and low. Therefore, and in order to avoid unneccesary animal testing, the study was carried out over only 15 days and with a reduced number of fish and water samples for analysis.
- We find it likely that the steady-state was reached during the uptake phase, since the measured chlorophene concentrations in fish (see listed concentrations above) indicate no increasing trend in either of the treatment groups.
- The REACH endpoint specific guidance, chapter R7c, section R.7.10.3.1, indicates that the uptake phase can be shortened: “After reaching an apparent steady-state concentration (or after 28 days, whichever is sooner), the remaining fish are transferred to clean water and the depuration is followed”.

<u>Main conclusion</u>

Even though the study has some deviations from the guideline, we think these deviations are justified and we are of the opinion that the main conclusion in the report is valid

("chlorophene is not expected to bioaccumulate in the environment"). We apologise for the lack of correct lipid-normalised BCF values in the CLH report. The correct lipid-normalised BCF values as presented above in this document are lower than the BCF values based on whole body weight, i.e. < 100 L/kg.

RAC's response

RAC thanks the DS for the clear interpretation of biodegradability test results.
Concerning bioaccumulation in fish, the detailed explanation and the introduction of the calculations by the DS were made clear that the correct lipid-normalised bioaccumulation value is only 55–56 L/kg, i.e. lower than the not normalised value of 107–110 L/kg and well below the threshold of 500 L/kg.
The test details shown in DS's response are convincing arguments in favour of accepting the study as valid, even though the study has some deviations from the guideline.

Date	Country	Organisation	Type of Organisation	Comment number
07.10.2014	Germany	Lanxess Deutschland GmbH	Company-Manufacturer	31

Comment received

General comments:

The following comments are related only to the classification derived from ecotoxicological and env. fate data. Comments on C&L for toxicological endpoints are filed separately.

For the ecotoxicological and env. fate endpoints Lanxess supports the following classification and labelling of chlorophene:

Aquatic Acute 1; H400
Aquatic Acute M-factor=1
Aquatic Chronic 1; H410
Aquatic Chronic M-factor=10

This classification proposal, where differing from the applicant's proposal, are supported by study data and sound scientific arguments in the related subsections of the commenting form.

Hazardous to the aquatic environment
Chlorophene meets the criteria for being classified as category Aquatic Acute 1 / H400 and Chronic 1 / H410.

However, for assessment of a multiplying factor (M-factor) an Early Life Stage (ELS) test with zebra fish (Danio rerio) with a reported NOEC (mortality) of 0.00058 mg/L is suggested by the Norwegian authorities.

The validity of this value is questionable. Usually the assessment of data quality includes the assessment of adequacy of the information for classification purposes and an assessment of both relevance and reliability.

While the data for the acute toxicity of chlorophene was quite in the range of similar data generated for other phenolic compounds, the data of this ELS Test generated at the CONFIDENTIAL test institute (test institute 1) in 2008 for chlorophene with Danio rerio (CONFIDENTIAL, 2008, A7.4.3.2/02) came out surprisingly low. In the test, a mean wet

weight per fish of 0.1246 mg and a mean length per fish of 0.533 cm were determined in the untreated control sample. In the treated samples, the mean wet weight per fish ranged between 0.1 and 0.3 mg and the mean length per fish ranged between 0.509 and 0.558 cm.

In order to better understand the criteria for a sound fish breeding, another CONFIDENTIAL test institute (test institute 2) was consulted. This test institute 2 is very experienced in fish breeding of the test species Danio rerio and confirmed that even the untreated control animals in the related study A7.4.3.2/02 performed by the test institute 1 suffered from reduced length and weight growth when compared to breed animals of the other test institute 2 with a mean wet weight per fish of 7.07 mg and a mean total length per fish of 0.90 cm (CONFIDENTIAL, 2011)* These values were recorded in two control monitoring replicates determined in test institute 2 using the same experimental setup as test institute 1.

Furthermore, the OECD technical guideline being updated in 2013 gives an indication of the typical minimum mean total length of 1.1 cm for Danio rerio which is twice as high as the mean length per fish of 0.533 cm as determined by the test institute 1.

Another peer reviewed publication by Roex et al. (2002) reported a mean wet weight per fish of 5.92 mg in the untreated control sample which is by a factor of 47 higher than those weights reported by the test institute 1.
In Table 1, the mean wet weight and mean length per fish values of the cited references are compared.

Table 1: Comparison of mean wet weight and length per zebrafish (*Danio rerio*) in untreated control samples.

Parameter	Test institute 1 (CONFIDENTIAL, 2008, A7.4.3.2/02)	Test institute 2 Statement (CONFIDENTIAL, 2011)	OECD TG 210 (2013)	Publication (Roex et al., 2002)
Wet weight [mg]				
Rep 1	0.1291	6.73	n.a.	n.a.
Rep 2	0.1200	7.42	n.a.	n.a.
Min	n.a.	0.2	n.a.	n.a.
Max	n.a.	20.5	n.a.	n.a.
Mean	**0.1246**	**7.07**	n.a.	**5.92 ± 2.54**[b]
Length [cm]				
Rep 1	0.545	0.90	n.a.	n.a.
Rep 2	0.520	0.91	n.a.	n.a.
Min	n.a.	0.5	n.a.	n.a.
Max	n.a.	1.4	n.a.	n.a.
Mean	**0.533**	**0.90**	**1.1**[a]	n.a.

[a] Typical minimum mean total length of control fish at the end of the study (cm), is not a validity criterion but deviations below the figure indicated should be carefully examined in relation to the sensitivity of the test. The minimum mean total length is derived from a selection of data available at the current time.

[b] Average fresh weight ± SD of zebra fish after 28 days in an Early Life Stage test, number of individuals: 20

When comparing the values as reported in the different sources (Table 1), it becomes relatively clear that the fish population used in the ELS test conducted at the test institute 1 in 2008 (A7.4.3.2/02) had very low mean wet weight and mean length values per fish.

Although the validity criteria of the test A7.4.3.2/02 were fulfilled and hatching of the fish larvae was very good with 100% after 9 days, the small fish weights and sizes reported at the test end (30 days after hatching) indicated that either poor keeping conditions or an unhealthy fish population must have interfered with the test results.

Considering the classification of chlorophene based on the NOEC being derived from a questionable test result with a probably unhealthy fish population and/or poor fish keeping conditions may lead to an overestimation of the M-factor. Therefore, we suggest selecting the acceptable test with Daphnia magna for classification and labelling, which also leads to a classification category Chronic 1 / H410, but to an M-factor of 10 as derived by the NOEC (reproduction) of 0.0067 mg/L.

On behalf of Lanxess Deutschland GmbH

CONFIDENTIAL, 2008, A7.4.3.2/02.
OECD, 2013. Test No. 210: Fish, Early-life Stage Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing. doi: 10.1787/9789264203785-en
Roex E et al, 2002. Sensitivity of the zebrafish (Danio rerio) early life stage test for compounds with different modes of action. Environmental Pollution 120 (2002) 355–362 (http://www.falw.vu.nl/nl/Images/177%20-%20roex_tcm19-29939.pdf)

* data have been submitted to the RMS Norway by Lanxess recently, i.e.
CONFIDENTIAL, 2011, Statement.

ECHA note: The following attachment was provided [Attachment 1]

Chlorophene - Comments on the dossier proposing harmonised classification and labelling submitted by Norway (Confidential attachment).

Dossier Submitter's Response

Thank you for your comment. We acknowledge the uncertainties around the weight and size of the fish based on the information currently available in the CLH report and the confidential Annex 2 to the report, and have therefore gathered more information in this response.

In the original dossier (documentation package) for the application of the use of chlorophene as a biocidal active substance in disinfectant products, an early life-stage study from 2007 was submitted. This study could however not be used in the risk assessment, since it could not be used to derive a NOEC. The reason for this was that the tested concentrations were too high, i.e. significant effects were observed at the lowest chlorophene concentration. It was therefore replaced by the early-life stage study A7.4.3.2 (confidential, 2008) which is described in the CLH report and for which a study summary is available in the confidential Annex 2 to the CLH report. This study was run with lower chlorophene concentrations enabling the derivation of a NOEC value.

We think the 2007 study could be used as additional information to support the NOEC of 0.58 µg/L from the 2008 study. The study summary for the 2007 ELS study was not part of the confidential Annex 2 to the CLH report, but it has now been uploaded in the confidential folder on CIRCABC as "fish_ELS_2007.doc".

Both the studies have been conducted according to OECD guideline 210, using zebrafish as the test organism. Both are GLP compliant studies. The test setup is similar in the two tests. The length of the fish at the end of the test in the 2007 study ranges from 0.759-0.846 cm (compared to 0.509-0.558 cm in the 2008 study), whereas the weight of the fish in the

2007 study ranges from 3.2-5.4 mg (compared to 0.1-0.3 mg in the 2008 study). In other words, the fish are larger in the 2007 study. However, the resulting effect concentrations are still comparable. For example, the LOEC values from the 2007 and 2008 study are within the same order of magnitude, i.e. 7.4 µg/L and 1.7 µg/L, respectively, based on mean measured concentrations. See the table below for a comparison of the two studies, with respect to both effect concentrations and other study details.

Study year	**2007**	**2008**
Test guideline and test organism	OECD 210, no deviations, RI 1 Same species (zebra fish) and source of fish in the two studies.	OECD 210, no deviations, RI 1 Same species (zebra fish) and source of fish in the two studies.
Test concentrations (µg/L)	Nominal: 9.5, 31, 98, 310, 1000 Measured: 7.4, 31, 94, 254, 940	Nominal: 0.95, 3.05, 9.77, 31.3, 100 Measured: 0.58, 1.65, 7.91, 22.9, 73
Recovery of test concentrations	78-100 %	54-81 %
Length and weight of fish at end of study	Length: 0.759-0.846 cm Weight: 3.2-5.4 mg	Length: 0.509-0.558 cm Weight: 0.1-0.3 mg
Controls (repl. 1 / repl. 2), hatching time	97 / 93 % after 5 d 97 / 97 % after 7 d 97 / 100 % after 9 d	13 / 43 % after 5 d 93 / 90 % after 7 d 100 / 100 % after 9 d
Controls, survival	Survival at hatching: 100 % Survival at end of study (30 d): 73 %	Survival at hatching: 100 % Survival at end of study (30 d): 81.7 %
Survival at comparable nominal test concentrations	9.5 µg/L, survival at hatching: 100 % 9.5 µg/L, survival after 30 d: 33 % 31 µg/L, survival at hatching: 100 % 31 µg/L, survival after 30 d: 43 % 98 µg/L, survival at hatching: 100 % 98 µg/L, survival after 30 d: 0 %	9.77 µg/L, survival at hatching: 100 % 9.77 µg/L, survival after 30 d: 6.8 % 31.3 µg/L, survival at hatching: 100 % 31.3 µg/L, survival after 30 d: 10 % 100 µg/L, survival at hatching: 100 % 100 µg/L, survival after 30 d: 0 %
LC_{50}	8.0 µg/L (nom.)	1.1 µg/L (measured)
NOEC	hatching success: 310 µg/L (nom.) / 254 µg/L (measured) mortality: < 9.5 µg/L (nom.) / < 7.4 µg/L (measured) body length: 31 µg/L (nom. and measured) body weight: 31 µg/L (nom. and measured)	hatching success: 100 µg/L (nom.) / 73 µg/L (measured) mortality: 0.95 µg/L (nom.) / 0.58 µg/L (measured) body length: 31.1 µg/L (nom.) / 22.9 µg/L (measured) body weight: 31.1 µg/L (nom.) / 22.9 µg/L (measured)
LOEC	9.5 µg/L (nom.) / 7.4 µg/L (measured) Effect: 67.2 %	3.05 µg/L (nom.) / 1.65 µg/L (measured) Effect: 74.6 %

There are some differences, e.g. the hatching seems to have been slower in the 2008 study than in the 2007 study. However, several of the effect concentration results are within the same order of magnitude (e.g. the LOEC values based on mean measured concentrations, as mentioned above).

Since effect concentration results from the two tests are comparable even though the fish in the 2007 study are larger, and since the tests have been run following the same OECD guideline, we are of the opinion that the small size and weight of the fish in the 2008 study should not have impacted the NOEC result. Based on the comparison between the two studies, we do not think it can be ruled out that a NOEC for mortality from the 2007 study (if lower concentrations had been used) could be within the same order of magnitude as the

NOEC for mortality from the 2008 study, on which the proposed classification is based.

Our opinion is thus that the 2008 study can be considered valid for classification purposes and that a chronic M factor of 100 is warranted.

RAC's response

The DS's response clarifies the data in the CLH report and the uncertainties around the weight and size of the fish can be assessed based on wider information. The additional study, fulfilling the fish size criteria, gives similar results than the questioned study – considering the identical concentration ranges in the two studies. So the opinion of RAC is, that the NOEC of 0.58 µg/L is acceptable, and therefore the M-factor of 100 is justifiable.

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Finland		MemberState	32

Comment received

The Finnish CA supports the proposed classification Aquatic Acute 1; H400, M=1 and Aquatic Chronic 1; H410, M=100 for Chlorophene.

Abiotic degradation
We agree with the conclusion that hydrolysis is not an important degradation way for chlorophene. As said in the CLH-report chlorophene is considered to be hydrolytically stable based on the preliminary hydrolysis test at the tested pH values (<10 % hydrolysis after 5 days), and therefore, no additional testing is required. In the CLH report estimated half-lives of chlorophene at pH 7 and 9 are presented based on the results obtained from the preliminary test. We are of the opinion that according to the guideline the preliminary test is conducted only in order to examine whether the substance is hydrolytically stable or not. The estimated half-lives of clorophene, which are based on a weak correlation coefficient of the hydrolysis slopes, might be misleading and should not be presented in the CLH proposal.

Biodegradation
We agree with the conclusions that chlorophene cannot be considered rapidly biodegradable.

Bioaccumulation
The dossier submitter has concluded that chlorophene is not expected to bioaccumulate in the environment based on the measured steady-state BCFfish value of 110 L/kg (whole body) obtained from the OECD 305 test. We don't quite understand the reasoning for not using lipid normalized (5 %) values as recommended in the OECD 305 test guidance. In this case, it might mean that the bioaccumulation criterion (>500) is met. However, there is a surprisingly big difference between the whole body and lipid normalized BCF values and it would be important to see the raw data behind the values (the actual lipid content of the fish and the weight). Would it be possible to report them as well in the CLH proposal? We also acknowledge that as the chlorophene is considered to be not rapidly degradable, the classification will not be affected whether bioaccumulation criterion is met or not.

Aquatic toxicity
The proposed acute classification Aquatic Acute 1; M factor of 1 is based on the algae ErC50 value of 197.2 µg a.i./L. There are no other valid acute tests available. For chronic hazard there are valid studies for all three trophic levels and the lowest NOEC value on which the classification proposal is based on is obtained for fish (0.58 µg a.i./L).
Since the substance is used as an algaecide, a presumption of algae being the most

sensitive trophic level would be logical. Yet for chronic toxicity where we have data on three trophic levels, fish is the most sensitive. This raises question on the validity of the algae test from which both the acute and chronic data originates. The used nutrient concentrations in the test medium were very high compared to the recommendation in the OECD 201 guideline and we think that there should be more discussion in the CLH report whether it has affected the sensitivity of the test.

Dossier Submitter's Response

Thank you for your comments and for stating your agreement with the environmental classification proposal.

Abiotic degradation
We see your point and we agree that it would have sufficed to use the study to state that chlorophene is hydrolytically stable.

Biodegradation
Thank you for your comment on the conclusion.

Bioaccumulation
There has been a misunderstanding regarding the lipid-normalised BCF, the BCF of > 1000 presented in the CLH report is in fact not the lipid-normalised BCF but the BCF in the lipid fraction. The recalculated lipid-normalised BCF is lower than the whole body BCF. Please see our response to comment no. 30 above (under the subheading "Lipid content and lipid normalisation" and "Size/weight of fish").

Acute aquatic toxicity
Regarding acute aquatic toxicity, we acknowledge the disadvantage of not having valid acute tests for classification purposes. The toxicity data was considered adequate for the purpose of the assessment of chlorophene as a biocidal active substance, and therefore no new data was required in that context. We think you have a good point in that the chronic studies show that fish are the most sensitive group, and that this gives rise to the question whether algae actually represent the most acutely sensitive trophic level and whether this uncertainty should be taken into account in the acute M-factor. Even though we cannot see that the practical consequences of the acute M-factor will be of notable significance for e.g. the classification of mixtures containing chlorophene, provided that the proposed chronic classification remains, we think this point should be raised in the further discussions. However, please see our response directly below on the validity of the algae study on which the acute classification is proposed based.

As you point out for the alga study (Egeler et al., 2006), the nutrient concentrations in the test medium were higher than recommended in the OECD 201 guideline. We do however not see this as a major deviation and still consider the results reliable. According to the OECD 201 guideline, the cultures should be allowed unrestricted exponential growth under nutrient sufficient conditions. It is mentioned as important that the initial biomass must be sufficiently low to allow exponential growth without the risk of nutrient depletion. Growth stimulation is discussed in point 59 of the guideline, and here it is stated that the addition of inorganic nutrients should not have any direct effect because the test medium should maintain a surplus of nutrients throughout the test. If chlorophene was expected to affect the availability of nutrients or minerals in the test medium, certain modifications to the procedure might have been appropriate. However, we have no reasons to expect interactions between chlorophene and the constituents of the test medium. The nutrient medium given in the test guideline is designed for an optimal sensitivity of the study and this should ideally have been used. However, since the deviation is that the nutrition

concentrations are higher and not lower than recommended in the test guideline, the requirements of nutrient sufficient conditions are certainly met. In conclusion, we consider the results of the alga study as valid.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
24.10.2014	Belgium		MemberState	33
Comment received				
No valid acute aquatic toxicity studies for chlorophene are available for fish and invertebrates. However based on the algae result(Pseudokirchierella subcapitata 72hErC50=0.1972 mg/l (mm)) the substance should be classified as Aquatic acute 1, H400. On the basis of this acute result an M-factor of 1 (0.1mg/l<LC50≤1mg/l) should be applied. The lowest NOEC for the three trophic levels was obtained in fish (Danio rerio 30dpost hatch NOEC=0.00058mg/l mg/l (mm)- mortality). The substance is not rapidly degradable and therefore it is justified to classify, following the classification criteria of regulation 1272/2008, as Aquatic chronic 1, H410 . In view of the proposed classification and toxicity band for chronic toxicity between 0.0001mg/l and 0.001mg/l and the fact that the substance is not rapidly degradable an M-factor for chronic toxicity of 100 should be assigned. In conclusion : we agree with the proposed environmental classification by the Norwegian Environmental Agency. Some editorial or/and minor comments : 5.3.1.2. Bioaccumulation : - Following OECD305 the uptake phase in the aqueous exposure test is usually run for 28 days. Was the steady state already achieved after 8 days? - For substances with high lipophilicity (i.e. with log KOW > 3), bioconcentration should be expressed as normalised to a fish with a 5% lipid content (based on whole body wet weight) in addition to that derived directly from the study. For Chlorophene this results in a BCF of 1130-1401 L/kg.				
Dossier Submitter's Response				
Thank you for your comments and agreements with the environmental classification proposal. **Bioaccumulation** There has been a misunderstanding regarding the lipid-normalised BCF, the BCF of > 1000 presented in the CLH report is in fact not the lipid-normalised BCF but the BCF in the lipid fraction. The recalculated lipid-normalised BCF is lower than the whole body BCF. Please see our response to comment no. 30 above (subheadings "Deviations from length of uptake and depuration phase, comment on steady-state" and "Lipid content and lipid normalisation").				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2014	Germany		MemberState	34
Comment received				

Chapter 5.4, table 42: In the column 'results' there is a typo. It should read ErC50 = 197.2 µg/L (algae) not mg/L. Bioaccumulation: We propose that the BCF related to fat content (BCF 1401 L/kg, 1130 L/kg) should be normalized to 5% lipid content (as suggested in REACH guidance Document, chapter R.7.10, p. 29). M-factor, acute toxicity: We wonder whether the M-factor of 1 is appropriate if only one acute endpoint is available, especially if there is no acute endpoint available for the most sensitive species fish. We consider if the M-factor for acute toxicity should be the same like the chronic M-factor.
Dossier Submitter's Response
Thank you for your comments. As you correctly point out, there is a misspelling in table 42. You are right that it should be E_rC_{50} = 197.2 µg/L = 0.197 mg/L. **Bioaccumulation** We have recalculated the lipid-normalised BCF, as there has been a misunderstanding regarding the terminology. The BCF of > 1000 is the BCF in the lipid fraction, it is not the lipid-normalised BCF as it might seem from the CLH report. Please see our response to comment no. 30 above (subheading "Lipid content and lipid normalisation"). **M-factor for acute toxicity** We agree with you in that there is an uncertainty in the acute M-factor due to only one valid acute endpoint. Please see our response to comment no. 32 above (subheading "Acute aquatic toxicity").
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2014	Sweden		MemberState	35
Comment received				
The Swedish CA supports classification of Chlorophene (Cas No 120-32-1) in Aquatic Acute 1 (H400) with an M factor of 1 and Aquatic Chronic 1 (H410) with an M- factor 100 as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard classes and differentiations based on valid data on acute toxicity to algae and long-term toxicity to fish and evidence of lack of rapid biodegradability of Chlorophene.				
Dossier Submitter's Response				
Thank you for your comments and agreements with the environmental classification proposal.				
RAC's response				
Noted.				

ATTACHMENTS RECEIVED

1. Chlorophene - Comments on the dossier proposing harmonised classification and labelling submitted by Norway (Confidential attachment). Submitted by Lanxess Deutschland GmbH on 07.10.2014.
   (Filename: 20141006_Comments_Classification_ENV_CONFIDENTIAL.pdf)
   [*Please refer to comments 3 and 31*]

2. Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section. Submitted by Lanxess Deutschland GmbH on 25.09.2014.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CLOROFENE; CHLOROPHENE; CLOROPHENE; 2-BENZYL-4-CHLOROPHENOL

(Filename: Chlorophene C+L Comments Lanxess_2014-09-24)
[*Please refer to comments 4, 9, 13, 24, 27*]