

Comments on the Proposal for Harmonized Classification and Labelling of Chlorophene - Human Health Section

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On behalf of

Lanxess Deutschland GmbH

Material Protection Products

Regulatory Affairs Business Line Actives & Disinfectants

Kennedyplatz 1, D-50679 Köln, Germany

4. Human Health Hazard Assessment

General Comments related to Human Health:

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.

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 sporadically 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 have 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

4.6 Repeated Dose Toxicity

Lanxess does not agree with the proposed classification with **STOT RE 1; H372 Causes damage to kidneys through prolonged or repeated exposure**.

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

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).

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

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 painting 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 malignant 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 malignant 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.

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 related 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

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