Cosmetics Europe
the personal care association

Comments on the CLH Report Proposal for Harmonised Classification and Labelling – Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2, Substance Name: EC number: 202-307-7 CAS number: 94-13-3; Dossier Submitter – Belgium (FPS Public Health, Food Chain Safety and Environment DGEM/ Department of Product Policy and chemical Substances / Management of Chemical Substances)

Headline summary

The Belgian REACH authorities submitted a classification proposal of Repr.2, H361fd for Propylparaben. This is mainly based on the results of an extended one generation reproductive toxicity study (EOGRTS) according to the OECD Test Guideline 443 after oral (gavage) administration at dose levels of 100, 300, and 1000 mg/kg body weight day to Wistar rats (2021). In the study report it was concluded that the NOAEL for developmental and reproductive toxicity, neurotoxicity and immunotoxicity was determined as 1000 mg/kg body weight/day. In contrast to that, the Belgian rapporteur defines a NOAEL of 300 mg/kg body weight/day based on changes in sperm motility and morphology. In addition to the proposed effects on sperm parameters in the EOGRTS, the rapporteur has cited decreased absolute anogenital distance (AGD) of male pups, and apparent increases in post implant loss as further reason for Repr.2 H361fd classification.

However, examination of the toxicology database for propylparaben demonstrates there are no toxicologically relevant effects on sperm, AGD or post implantation loss.

Given the definite lack of toxicologically relevant effects on sexual function and fertility, or on development, it is the opinion of Cosmetics Europe (CE) reproductive toxicology experts that there is no justification for a Repr.2 classification for propylparaben.

Fertility

According to the submitted CLH report, the 2021 EOGRTS revealed a reduction in sperm motility in the high dose groups of both the F0 and F1 generations. The CLH also states that the total number of abnormal sperm was greatly increased in the high dose groups of both generations, primarily driven by increases in the number of 'tail only' sperm. The conclusions from the CLH contrast with that of the study report which states that 1000 mg/kg/day was a clear reproductive NOAEL. The CLH conclusions also differ from the conclusions of reproductive toxicology experts from CE who concur that a NOAEL of 1000 mg/kg/day was established in the EOGRTS due to a clear lack of significant and adverse effects on reproduction. Moreover, the SCCS evaluated this study in its recent opinion on propylparaben (SCCS/1623/20, adopted on 30-31 March 2021) and agreed that the results of the EOGRTS suggest a NOAEL of 1000 mg/kg/day.

In the F0 generation, mean motile sperm counts were reported as 77.05% and 72.67% in the control and high dose (HD: 1000 mg/kg/day) groups respectively. In the F1 generation, the mean motile counts were reported as 79.10% and 72.42% in the control and HD groups respectively. However, in the view of CE reproductive toxicology experts these small, statistically not significant, decreases are not toxicologically relevant when compared with the historical control data (HCD) for motile counts of the conducting laboratory, which range between 65.25% to 98.17% (mean -/+ 2SD). This view is supported by the recent SCCS opinion (SCCS/1623/20).

In addition, examination of the individual animal data (IAD) reveals one outlier in the HD group of the F0 generation. Animal 92 has no motility data across all recordable fields (motile, static, and rapid counts) and 74% of the 200 sperm counted from this animal had 'abnormal morphology', a clear outlier of the group. Due to the extreme results across several parameters, compared to the remaining animals of the same dose group, CE reproductive toxicology experts consider it appropriate to exclude this animal from further analysis on a weight of evidence basis. Exclusion of this animal changes the mean motile count from 72.67% to 75.17% for the F0 HD group. In conclusion, despite small incremental decreases in mean values between HD groups and concurrent control groups, CE reproductive toxicology experts do not agree these constitute significant or toxicologically relevant changes to sperm motility.

The CLH report also highlights increases in the total number of abnormal sperm in both the F0 and F1 males. In the F0 generation, the mean total of abnormal sperm is reported as 8.25% and 13.33% in the control and HD groups respectively. However, as previously mentioned there is a clear outlier (animal 92) within the HD group with unusually divergent results across several parameters. Without this animal the total number of abnormal sperm is only 7.48% in the HD group, lower than the concurrent controls. In addition, the percentage of 'tail only' sperm are reported as 2.96% and 8.17% in the control and HD groups respectively, compared to only 2.96% and 2.83% when animal 92 is excluded.

Similarly, in the F1 generation when a clear outlier (animal 298) is excluded, the total number of abnormal sperm is 10.35% and 12.29% in control and HD animals compared to 10.35% and 19.06% when animal 298 is not excluded. In the 'tail only' analysis the values are 3.85% and 5% in control and HD groups without the outlier, compared to 3.85% and 11.17% including the outlier.

In summary, all the high dose values for total number of abnormal sperm are within the conducting laboratory HCD, which ranges between 0% to 19.30% (mean -/+ 2SD). Therefore, in conclusion, despite small incremental changes of some mean values between the HD

groups and concurrent control groups in the EOGRTS, CE reproductive toxicology experts do not agree these constitute toxicologically relevant changes of sperm morphology. It should also be noted that there were no changes in sperm parameters, fertility or development in the F0 parental or F1 cohort 1A. Moreover, the rapporteur use data from the study of Oishi (2002, incorrectly referred to in the CLH report as Oishi, 2012) to support their conclusions (see CLH Report, Table 34). But it has to be noted that the Oishi (2002) study was a non-GLP, non-guideline study with small group size. There were a number of control values in parameters that were well outside of the normal range. The data were not consistent with literature data and data from other studies of Oishi for daily sperm production (DSP), epididymal sperm counts and testosterone concentration, and there was no dose-response for the effect on DSP. In addition, a full study protocol and raw data are no longer available which makes the results irreproducible and thus, scientifically unreliable (Snodin, 2017).

The rapporteur should also have referred to three other good quality, reliable GLP studies, i.e., Hoberman et al. (2008), Sivaraman et al. (2018) and Gazin et al. (2013). None of these studies showed any evidence of adverse effects on the male reproductive system including sperm parameters and measurement of hormone concentrations. The NOAEL of all these well-powered GLP studies was 1000 mg/kg bw/day.

Development

According to the CLH report there were consistent effects on post implant loss across multiple studies, and a decrease in male AGD in the EOGRTS following treatment with propylparaben. The conclusions in the CLH contrast with that of both the OECD Test Guideline 414 and EOGRTS study reports which state 1000 mg/kg/day as a clear developmental NOAEL. The CLH conclusions also differ from the conclusions of CE reproductive toxicology experts who concur that a clear developmental NOAEL of 1000 mg/kg/day has been established due to a lack of significant and adverse effects on development. This view is supported by the recent SCCS opinion (SCCS/1623/20).

Male AGD, the distance between the anus and the external genitalia, is an androgen-sensitive endpoint of the masculinization. Regarding the AGD, all the evaluated values were in the range of historical control data and no dose dependency could be observed. Furthermore, the changes were not statistically significant and could not be revealed in both generations of the EOGRTS indicating that these effects are due to biological variability rather than test item related.

In the EOGRTS the AGD of the F1 males was 2.84mm and 2.71mm in the control and HD groups respectively, which looks like a statistically significant difference. However, the value of 2.71mm is well within concurrent HCD for this finding at the conducting laboratory (mean

of 2.6mm from 2073 male pups) and therefore although statistically significant, there is no biological or toxicological relevance to this change. Furthermore, the relative AGD measurements were not statistically different and are also well within HCD. This is similar in the F2 males, where the AGD was 2.98mm and 2.77mm in the control and HD groups respectively. Again, although this change is recorded as statistically significant there is no biological or toxicological relevance to it as the HD value (2.77mm) is well within the concurrent HCD for AGD. In conclusion, despite small incremental changes to some mean values between the HD groups and concurrent control groups, CE reproductive toxicology experts do not agree that these constitute toxicologically relevant changes on AGD or development.

A decrease in AGD in male offspring may be associated with genital malformations at birth and reproductive disorders in adulthood. A concern regarding the reduction of AGD in male and female pups was raised by the CLH-dossier submitter based on the data provided in the OECD Test Guideline 443. Based on the data from the EOGRTS in male pups from the parental generation, on PND 0 marginal shorter absolute but not relative AGD was observed only in the HD group (1000 mg/kg bw per day) when compared to the concurrent controls. It is important to note that AGD is influenced by the body weight of the animal and therefore, this needs to be taken into account when evaluating the data (OECD Guidance document No. 151) and a normalization using the cube root of body weight is recommended in Test Guideline 443. In case of propylparaben (parental generation) no statistically significant effect could be observed after normalization of AGD to cube root of body weight. More importantly, no dose dependency was observed in these effects and all the values were well within the range of historical control data revealing that this effect is not considered to be test item related but due to biological variation.

Due to the strong correlation between AGD and various reproductive disorders and malformations, an isolated consideration of AGD is not appropriate. Especially in the case of propylparaben, where changes in male AGD were only minimal, well within the range of historical control data and not dose dependent, the concurrent lack of any functional or histopathological impairment contradicts the assumption of an adverse effect. The results from the EOGRTS clearly demonstrate that *in utero* exposure to propylparaben up to the limit dose of 1000 mg/kg bw per day did not induce any morphological or histopathological abnormalities in male reproductive organs. Importantly, functional parameters such as fertility and mating index were also not affected after treatment with propylparaben up to 1000 mg/kg bw per day neither in parental, nor in F1 and/or F2 animals. The effects on nipple retention did not support a possible relationship of the AGD findings with an anti-androgenic mode of action as the nipple retention is upregulated in the parental but downregulated in the F1 generation. These deviating and, in particular, contradictory effects, scientifically support the view that an anti-androgenic mode of action can be excluded and that the minor

changes on AGD (and nipple retention) can plausibly be regarded as being due to biological variability and possible impact of body weight changes and therefore, are not considered to be a toxicologically relevant effect.

With regard to post implant losses, in both the EOGRTS and OECD Test Guideline 414 studies the small incremental changes in these values were not dose responsive, not statistically significant and therefore, not considered toxicologically relevant. In two lower powered range finding studies, there was an apparent increase in what appears a dose responsive manner, however the values for post implant loss were all well within the conducting laboratory HCD for this finding. The CLH states for one range finding study that post implant loss of 12.4% at the HD is 'severely higher' than the concurrent control group (at 5.9%), however the mean post implant loss in historic control animals is comparable to the HD group at 10.1%, with a range of values from 0- 51.8% -/+ 2SD. In conclusion, despite small incremental changes to some mean values between the HD groups and concurrent control groups, CE reproductive toxicology experts do not agree that these constitute toxicologically relevant changes of post implantation loss or development.

For the purpose of weight of evidence, the CLH should have also referred to the study of Sivaraman et al. (2018) which showed no evidence of adverse effects on development, including no increase in post implant loss. The NOAEL of this well-powered GLP study was 1000 mg/kg bw/day.

Other Relevant Information

Following oral exposure, propylparaben is very rapidly metabolised to p-hydroxybenzoic acid which is cleared within 4-6 hours via urinary excretion in humans. At the oral doses administered (up to 1000 mg/kg bw in rats and up to 2 mg/kg bw in humans) excretion is principally urinary and fast with more than 90% of the propylparaben dose excreted within 24 h post-dosing in both rat and human, confirming that propylparaben does not accumulate in the body (Shin et al., 2019). The rapid metabolism of propylparaben, particularly in the rat, substantiates that after oral administration no plasma levels are achieved which may be high enough to lead to adverse effects such as, e.g., reproductive toxicity.

Comparison with the CLP criteria

Substances suspected of being toxic for human reproduction are classified in category 2 for reproductive toxicity, i.e., when there is some evidence from humans or experimental animals, possibly supplemented with other information, of **an adverse effect on sexual function and fertility, or on development.**

The CLH concludes that due to severe effects in sperm in the absence of clear general toxicity, a classification as Repr. 2 H361f is warranted. It also concludes that classification as Repr. 2, H361d is warranted based on AGD and post-implantation loss's modifications. **However, examination of the toxicology data demonstrates there are no toxicologically relevant effects on sperm, AGD or post implant losses.**

Given the definite lack of toxicologically relevant effects on sexual function and fertility, or on development, it is the opinion of CE reproductive toxicology experts that there is no justification for a Repr.2 classification for reproductive toxicity of propylparaben.

Conclusion

A classification for reproductive and developmental toxicity according to CLP is not justified based on the scientific evidence in the context of the regulatory criteria (REGULATION (EC) No 1272/2008). According Regulation (EC) 1272/2008 of the European Parliament and of the Council („CLP Regulation"), classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence which means that all available information that bears on the determination of reproductive toxicity is considered together in that both, positive and negative results are assembled together into a weight of evidence determination.

For an effect to warrant classification, CLP criteria primarily require, that the effect is adverse, which is furthermore characterized by several additional criteria including the assessment of the biological and toxicological significance, as well as the nature, severity, and incidence of the effect. Furthermore, conclusions on the inherent ability of a chemical to induce a specific adverse effect should be based upon the available data and an assessment of total weight of evidence which includes assembling together both positive and negative results. As already described, the extensive scientific evidence from animal studies involving oral exposure to propylparaben demonstrates a lack of adverse reproductive effects per the CLP criteria and therefore classification for development and fertility effects is not required. To conclude on a classification determination, there is a need to take into account the whole toxicological evidence for propylparaben in a robust weight of evidence approach to develop an informed regulatory decision that is commensurate and proportionate with all available data. Following these principles, the following can be concluded with regard to the concerns brought forward by the CLH-dossier submitter: an isolated consideration of effects on single endpoints which are lacking statistical significance, and which are without any dose dependency, is not appropriate and/or justified for classification as developmental and reproductive toxicity. More importantly, all findings of concern discussed by the evaluating MS Belgium are well within the range of the historical control data and thus, represent biological variation rather

than a substance-related true effect of toxicological relevance. Additionally, functional parameters which are correlated to single values need to be considered in order to assess the adversity of effects. When taking the complete set of available toxicological data into account, no adverse effect on all above mentioned functional developmental and reproductive parameters could be observed up to the limit dose of 1000 mg/kg bw per day and thus, a classification as Repr. Cat 2 is not justified.