EUROPEAN CHEMICALS AGENCY

Helsinki, 9 July 2018

Addressee: [redacted]

Decision number: CCH-D-2114412038-60-01/F
Substance name: Methyl 4-hydroxybenzoate
EC number: 202-785-7
CAS number: 99-76-3
Registration number: [redacted]
Submission number: [redacted]
Submission date: 25/02/2015
Registered tonnage band: 100-1000

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance;**

2. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance;**

3. **Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - At least two weeks premating exposure duration for the parental (P0) generation;
   - Dose level setting shall aim to induce some toxicity at the highest dose level;
   - Cohort 1A (Reproductive toxicity);
   - Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation;
   - Cohorts 2A and 2B (Developmental neurotoxicity); and
   - Cohort 3 (Developmental immunotoxicity).

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI to the REACH Regulation. To ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective annex, and adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by ***16 July 2021***.

You shall also update the chemical safety report, where relevant.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Authorised[1] by **Ofelia Bercaru**, Head of Unit, Evaluation **E3**

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

A "sub-chronic toxicity study (90 day)" is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided a key study record for a "repeated dose 28-day oral toxicity study" (test method: OECD TG 407). However, this study does not provide the information required by Annex IX, Section 8.6.2., because exposure duration is less than 90 days and the number of animals per dose group is significantly lower. Therefore, the sensitivity of a 28-day study is much lower than that of a 90-day study.

You have additionally provided study summaries of five studies which are marked as weight of evidence. These studies are of low reliability and you have assigned them Klimisch score 3 or 4. In view of this low reliability, ECHA cannot consider these as reliable evidence. You have not provided a justification for the weight of evidence, as required by Annex XI, 1.2 of the REACH Regulation. Consequently ECHA concludes that there is no evidence on why and how these studies, altogether, could be considered as reliable information for assessing of sub-chronic toxicity. Thus, the adaptation of Annex XI, 1.2 does not fulfill the information requirement.

Following the notification of the draft decision you submitted comments disagreeing to the request for a 90-day study. In your comments you argued that there are already six repeated dose studies with methyl 4-hydroxybenzoate performed in rats, dogs, guinea pigs and rabbits and that the key 28-day study showed no systemic effects, which was in line with the outcome of the other supporting studies. You also argued that "*The referred chronic and subchronic studies exhibit minor shortcomings mainly in the reporting and documentation due to older study designs and reporting styles. However, all tests have been conducted, according to accepted scientific criteria regarding meaningful and informative endpoint investigations (i.e. haematology, clinical chemistry, histopathology etc.) and thus, the results are considered to be not only valid for use in a WoE approach but especially informative regarding species extrapolation considerations.*" You argue that "*the absence of systemic effects of methyl 4-hydroxybenzoate is sufficiently proven.*" ECHA understands that you are making a Weight of Evidence (WoE) argument for all six studies. ECHA notes that the five studies provided for the WoE approach in the dossier are non-GLP and non-guideline studies, and you assigned them with a reliability score of Klimisch 3 or 4. Klimisch 3 is assigned to a study which is not reliable, and Klimisch 4 to a study where it is not possible to assess the reliability of the study. Annex XI, 1.1.2 provides that non-GLP and non-guideline studies may be acceptable provided that the listed criteria are met, and this includes that "adequate and reliable documentation of the study is provided." However, you have not provided a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report. For these five studies, there is not a detailed description of the methods at a level comparable to the

OECD Test Guideline OECD 408. There is thus not adequate and reliable documentation present in order for ECHA to be able to make an independent evaluation of these studies.

Nonetheless, ECHA considers that, for the five studies provided for the WoE approach in the dossier, these studies have major deficiencies in providing adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3). Specifically, ECHA notes that in the three supporting sub-chronic studies with rabbits, guinea pigs and rats, the clinical biochemistry, urinalysis, organ weight, gross pathology and histopathology were not examined. In the chronic toxicity study in rats ([redacted] 1956), haematology, clinical chemistry and urinalysis were not examined and there are no data on clinical signs and organ weights. In the chronic study in mongrels ([redacted] 1956), there are no data on clinical chemistry and organ weights and only kidney, liver, heart, lung, spleen and pancreas were listed as subject to histopathological examination (versus 30 organs specified in OECD 409). There are no statistics provided in any of the five WoE studies. In addition, in the chronic studies only 6 animals/sex/dose ([redacted] 1956, rats) and two or three animals/group (sex not specified, [redacted] 1956, mongrel dogs) respectively were used. The OECD 452 on Chronic Toxicity Studies specifies that "*For rodents, at least 20 animals per sex per group should normally be used at each dose level, while for non-rodents a minimum of 4 per sex per group is recommended*." ECHA rejects your contention that these studies have 'minor shortcomings' and considers that there are major defects in the coverage of key parameters for these studies. There is no adequate explanation of how these major defects are addressed in your weight of evidence argument.

The key study was performed according to OECD 407, and as a 28-day study, does not provide the information required for a sub-chronic study which is 90-days in duration. In view of the major defects in the five weight of evidence studies, ECHA considers that there is not a sufficient weight of evidence from considering the 28-day study together with the additional five studies to provide reliable information about exposure over a duration of 90 days.

You have also argued that the absence of systemic effects of methyl 4-hydroxybenzoate is sufficiently proven from this weight of evidence. ECHA notes that in the 28-day study summary in dossier it is stated "*One male and one female at 1000 mg/kg/day were sacrificed for ethical reasons on Day 14 and 24 respectively, showing several clinical signs indicative of ill health. Microscopic findings examination revealed minimal/slight erosions in the stomach, correlating to the irregular surface recorded at necropsy, slight red pulp atrophy of the spleen and slight/moderate lymphoid atrophy of the thymus, correlating to the reduced size recorded at necropsy. Since these deaths occurred in the highest dose group, and based on clinical signs in the surviving animals at 1000 mg/kg, a relation to treatment with the test substance could not be excluded*." ECHA considers that there is evidence of severe toxicity, including death, and that these effects are substance related; ECHA rejects your contention that these effects were "not considered substance related" and that there is an absence of systemic effects. Furthermore, although the available repeated dose studies provide evidence of relatively low toxicity, there is inconsistency between the histopathology findings of 28-day key study and the two chronic toxicity studies (with NOAELs of 5.5 and above 1g/kg). In the 28-day key study, clear signs of organ toxicity were observed, while in the chronic studies, at much higher dose levels no histopathological effects were seen. The 28-day study is a recent GLP study performed according to the testing guideline and therefore deemed more reliable. This inconsistency is evidence that there is not a consistent WoE for an absence of systemic effects, and it is not addressed in the arguments for WoE.

Therefore, in the light of the arguments above, it is not possible to conclude that there is sufficient weight of evidence leading to the assumption that this substance has or has not a particular dangerous property. Consequently, the WoE adaptation cannot be accepted. As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6, July 2017) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is reported to occur as a white crystalline water soluble solid with low vapour pressure. Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.

According to the test method EU B.26./OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

Notes for your consideration

ECHA notes that a revised version of OECD TG 408 may be adopted later on this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. After the adoption of the revised version of the OECD TG 408 you should test in accordance with that version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

Even if you start testing before the guideline is published, it is appropriate to consider including these endocrine-sensitive parameters in your testing protocol in accordance with the proposed revised version of the draft guideline (see http://www.oecd.org/env/ehs/testing/section4-health-effects.htm).

2. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained.

ECHA notes that the registration dossier contains negative results for both these information requirements. Therefore, adequate information *on in vitro* gene mutation in mammalian cells needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of an *in vitro* gene mutation study in mammalian cells in the dossier that would meet the information requirement of Annex VIII, Section 8.4.3.

Following the submission of the draft decision you submitted comments disagreeing with the request for an *in vitro* gene mutation study in mammalian cells. In your comments you argued that "*Only from a pure formal point of view this requirement might be justified but due to the fact that there are several valid Ames tests, in vitro chromosome aberration studies as well as in vivo genotoxicity studies (dominant lethal assay, chromosome aberration study) performed with methyl 4-hydroxybenzoate available, the additional value of a newly conducted in vitro gene mutation assay in mammalian cells is highly questionable.* "

ECHA notes that neither the available negative Ames nor the in vitro and in vivo cytogenicity tests can fulfil the endpoint requirement for a gene mutation study in mammalian cells. The available *in vivo* rodent dominant lethal test is not suitable to fulfil for this standard information requirement as this test only indicates that the substance has affected germinal tissue of the test species but does not provide information on gene mutation events in somatic cells. Furthermore, although gene mutations and toxic effects cannot be excluded, dominant lethals are generally accepted to be the result of chromosomal aberrations, such as structural and numerical anomalies, and so do not provide relevant information for gene mutation.

During the comenting period you provided a study report for a gene mutation assay in Chinese hamster V79 cells in vitro with propylparaben. You have justified the read-across to this analogue substance with the following argument: "*Since ECHA commonly performs read across approaches between the alkyl 4-hydroxybenzoates to derive potential (common) mode of actions of the single paraben substances, we are of the opinion that a read across from the newly performed HPRT test with propyl 4-hydroxybenzoate to fill the data gap identified for methyl 4-benzoate is fully sufficient, appropriate as well as scientifically and legally justified.*" According to Annex XI, 1.5, you must provide adequate and reliable documentation of the applied method, which must include a specific justification whereby relevant human health properties of the registered substance may be predicted from data for the source substances. We assume that your arguments is based on ECHA assessment of the compliance of information submitted under Annex IX, 8.7.3. In that context, ECHA argued that under Annex IX/X, 8.7.3 a condition is to take into account "*existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action*". You seem to consider that this condition is equivalent to a read across justification. However, this condition is based on a specific requirement set out in Column 2 of this provision and is different from the requirement for valid read across justifications set out in Annex XI, 1.5. Accordingly, your dossier does not address why prediction of the human health properties would be possible. In the absence of this information, ECHA cannot verify that the properties of the registered substance can be predicted from the data on the source substance.

Hence, you have not established that relevant properties of the registered substance can be predicted from data on the analogue substance. Since your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5., it is rejected and it is necessary to perform testing on the registered substance.
As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490)

3. Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex IX of the REACH Regulation, if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD TGs 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity.

If the conditions described in column 2 of Annex IX are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3.

Further detailed guidance on study design and triggers is provided in ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6.0, July 2017).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information requirement

ECHA considers that concerns in relation with reproductive toxicity within the meaning of column 1 of section 8.7.3., Annex IX of the REACH Regulation are observed. More specifically, in a 56-day repeated dose dietary study ([redacted] 2005, reliability 1, non-guideline GLP) with methylparaben (the registered substance subject to this decision) administered *ad libitum* to male rats in doses of 100 ppm, 1000 ppm, 10000 ppm (equivalent to 11.2, 110.0 and 1141.1 mg/kg bw/d), *"The number of normal sperm was significantly reduced (p</=0.05) and corresponding values for percent abnormal were significantly increased in the 1000 ppm (p</=0.01) and 10000 ppm (p</=0.05) exposure groups. The percent abnormal sperm in these groups (mostly composed of sperm with no head) was significantly increased compared to the control group values."*. Although it is argued that *"These differences were not related to Methylparaben because the values were not dosage-dependent."*, ECHA notes that for both groups the effects were of a similar type and were both significantly different compared to control.

Moreover, there are indications of endocrine disruption properties stemming from a study by Vo et al. (2010)[2], which is not present in the dossier. In this study it was shown that parabens (including methylparaben) can produce suppressive effects on hormonal responsiveness, e.g. by reducing the serum levels of estradiol and thyroxine (T4), and they

[2] **Vo et al. 2010** (Vo TT, Yoo YM, Choi KC, Jeung EB. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reprod Toxicol. 2010 Jun;29(3):306-16.

can disrupt the morphology of reproductive target tissues such as ovaries. Also a delay in the date of vaginal opening in rats was observed in methylparaben-treated groups.

Taking into account the above mentioned concerns in relation to reproductive toxicity, pursuant to Annex IX, Section 8.7.3. Column 1 an extended one-generation reproductive toxicity study is thus an information requirement for registrations of the registered substance.

Furthermore, ECHA notes that one member of the joint submission has a tonnage band of more than 1000 tonnes per year entitling to information requirement of Annex X.

Following the submission of the draft decision you submitted comments disagreeing with the request for an extended one generation study. In your comments you argued that:

"according to column 1 of section 8.7.3, Annex IX of REACH Regulation the "extended one-generation study ... is required, if the 28-day or 90-day study indicates adverse effects on reproductive organs or tissues." There are no results of available studies performed with methyl 4-hydroxybenzoate which would trigger the conduct of such an animal-consuming study." You then argue that in respect of the 28-day study of [redacted] (2005, 2008), there was not a reduction in sperm number nor an increase in the number of abnormal sperm.

In respect of sperm number, you state, "*However, in the respective publication ([redacted] et al. 2008) it is clearly stated that "exposure to methylparaben at all dose levels (up to 10000 ppm in diet) did not affect sperm motility, sperm count or daily sperm production". The concrete values on sperm count given in the publication are 875±379 million/g for the control group and 662±423 million/g for the low, mid and high dose respectively.*" However, in your dossier you state that there is a statistically significant reduction in sperm count in this study. You do not explain why the information in your dossier is wrong, or that it is wrong. ECHA considers that this effect (in the lowest dose group) strengthens the concern for adversity seen with the abnormal sperm finding.

In respect of abnormal sperm, you argued that while ECHA found a concern such as a reduction of normal sperm in the [redacted] 2005 study, "*The authors very generally reported that methyl 4-hydroxybenzoate exposure resulted in a significantly higher incidence of abnormal sperm in all dose groups;*" and that "*The original report of this GLP study ([redacted]) includes a more detailed description of the findings available which has allowed the study director to conclude that observed differences in the sperm evaluation part of this study "were not considered related to methylparaben because the values were not dosage-dependent. In addition, extensive histopathology of the reproductive organs with detailed qualitative examination of the testes including the tubular stages of the spermatogenic cycle did not reveal any adverse findings. The no-observable-effect level (NOEL) for general toxicity for methyl 4-hydroxybenzoate including histopathology of reproductive organs and sperm analysis was 10000 ppm (in the diet). No effect occurred at this highest dose tested".*

The studies ([redacted] (2005) and Vo et al. (2010)) mentioned in the draft decision raise concerns in relation with reproductive toxicity that need clarification, and so an extended one-generation study (EOGRTS) is required. In particular, the study of [redacted] (2005) published later as [redacted] et al.(2008), showed that "*Methylparaben exposure resulted in a significantly higher incidence of abnormal sperm in the 1,000- ($p \leq 0.01$) and 10,000- ($p \leq 0.05$) ppm exposure groups. These were mostly composed of sperm with no head. Although the incidence was statistically significant, its magnitude was only 4–5 vs. 2.3% in*

controls." The authors did not believe that this was treatment-related. Nevertheless, ECHA considers that a treatment related effect cannot be excluded based on this data, that there is statistically-significant effect in the top two dose groups, that this is dose-related and that this is an adverse effect on a reproductive organ.

In your comments you also made reference to the study of Vo et al. (2010): *"Regarding the cited study from Vo et al. (2010) it has to be stressed that most effects reported are attributed to longer-chain parabens other than methyl 4-hydroxybenzoate. (...) The undifferentiated generalization of effects observed for parabens with longer alkyl chains is not supported by any regulatory expert panel and separate evaluations are recommended"*.

ECHA notes that the study of Vo et a. 2010, raises additional concerns, including ones related to thyroid effects. You argued that *"most effects"* reported are attributed to longer-chain parabens other than methyl 4-hydroxybenzoate. However, in the paper, the results for each paraben are given. The methyl paraben data (Table 4 in publication) show reduction in the serum levels of estradiol (albeit not significant) and of thyroxine (T4)(statistically significant). Also the specific data on methyl paraben show a significant delay in the date of the vaginal opening, a significant decrease in the number of 4 days cycle, a decrease in the weight of ovaries and changes in the weights of the kidney, liver, adrenal gland, and thyroid gland. These effects are specifically reported for methyl paraben and ECHA considers that these effects are "other concerns in relation with reproductive toxicity", which is the condition of column 1 of Annex IX, 8.7.3. After considering your comments on the concerns triggering the EOGRTS, ECHA considers that the concerns remain, and so an EOGRTS is triggered.

You claim that *"none of the members of the joint submission has a tonnage band of more than 1000 tons per year and therefore, the requirements pursuant to Annex X do not apply for methyl 4-hydroxybenzoate."* However, as already indicated in the Appendix 2, one of the members of the joint submission, has a tonnage band above 1000 tpa. Therefore, on this basis also an extended one generation-study is needed.

You argue that since an extended one generation study is not triggered, then there is no obligation to provide data on developmental neurotoxicity or immunotoxicity. However, ECHA considers that there is a requirement for an extended one generation study, and that data on developmental neurotoxicity must be provided based on the reasoning for Cohorts 2A and 2B.

Finallly, you argue that there should be a testing strategy for methyl 4-hydroxybenzoate. You consider firstly that all toxicological endpoints are sufficiently covered, but ECHA disagrees for reasons as set out above. You state that there are final decisions which require you to provide information on propyl 4-hydroxybenzoate, and you propose to first await the results of these studies, and then identify if there are discrepancies in individual toxicological endpoints and/ or the overall toxicity profiles. You would then propose no testing on the basis of a read-across, or alternatively reconsider additional testing with the registered substance. ECHA rejects your proposal for a testing strategy for the following reasons. (a) For the endpoints concerned by this decision, you have not used adaptation by grouping and read-across as an adaptation in your registration dossier (b) you have proposed adaptation by grouping and read-across in this comment, for the single endpoint of in vitro gene mutation (see above). However, you did not provide a valid justification for the grouping and read-across adaptation according to Annex XI, 1.5, and ECHA rejects this adaptation for this endpoint. (c) there is currently no valid adaptation by grouping and read-across according to Annex XI, 1.5 for the relevant endpoints for this dossier, which could be used to form the basis for an acceptable strategy (d) ECHA considers that the results from

future or ongoing studies on propyl 4-hydroxybenzoate are not known now. It is not possible to conclude now that these studies would change the information required for the registered substance methyl 4-hydroxybenzoate. Accordingly ECHA cannot take this proposed strategy into account.

Information provided

You have sought to adapt this information requirement according to Annex XI, Section 1.2. of the REACH Regulation on weight of evidence. You provided the following justification for the adaptation:

"In accordance with Section 1.2 of REACH Annex XI, there is sufficient weight of evidence from several independent sources of information leading to the conclusion that Methylparaben does not cause toxicity to reproduction and thus does not have to be classified, because

- Methylparaben caused no irreversible or severe systemic toxic effects in a 28-day oral gavage study in rats (NOAEL 250 mg/kg/day) especially to reproductive organs and did not influence the oestrus cycle,

- Methylparaben did not cause any maternal or developmental effect in developmental studies in rats, mice and hamsters

- Methylparaben did not induce effects on reproductive organs and had no influence on sperm parameters and testosterone, LH/FSH blood levels when applied up to 1000 mg/kg bw/d to male rats in a 56 day dietary study

- Methylparaben did not show any significant effect in the uterotrophic assay up to 800 mg/kg bw/d

- Methylparaben does not have to be classified as skin sensitizing or as skin or eye irritating, indicating its very low tendency to interact with living cells and tissue.

It can therefore be concluded with sufficient certainty that Methylparaben will not cause toxicity to reproduction and that testing is not scientifically necessary. "

ECHA has evaluated your adaptation with respect to this provision. To support your weight of evidence adaptation you have provided the following sources of information:

- Key study: Repeated Dose 28-Day Oral Toxicity in Rodents, rat, oral route (OECD TG 407; GLP) with the registered substance, by [redacted] 2009 (study report [redacted]), reliability (rel.) 1.
- Key study: equivalent or similar to Prenatal Developmental Toxicity Study, rabbit, oral route (OECD Guideline 414, non-GLP) with the registered substance, [redacted] 1973, study report [redacted] rel. 2.
- Supporting study: equivalent or similar to Prenatal Developmental Toxicity Study, rat, oral route (OECD Guideline 414, non-GLP) with the registered substance, [redacted], 1972 (study report [redacted]), rel. 2,

- Supporting study: equivalent or similar to Prenatal Developmental Toxicity Study, mouse, oral route (OECD Guideline 414, non-GLP) with the registered substance, [redacted] 1972 (study report [redacted]), rel. 2
- Supporting study: equivalent or similar to Prenatal Developmental Toxicity Study, hamster, oral route (OECD Guideline 414, non-GLP) with the registered substance, [redacted] 1972 (study report [redacted]), rel. 2
- Weight of evidence study: in rat, oral route, non-guideline, non-GLP, with the registered substance, [redacted] 2004, rel. 2
- Weight of evidence study: in rat, oral route, non-guideline, non-GLP, with the registered substance, [redacted] 2005, rel. 1
- Weight of evidence study: in rat, oral, Uterotrophic Bioassay in Rodents according to OECD 440, non-GLP, with the registered substance, [redacted] 1998, rel. 2
- Weight of evidence study: in mouse, oral, Uterotrophic Bioassay in Rodents according to OECD 440, non-GLP, with the registered substance, [redacted] 1999, rel. 2
- Weight of evidence study: in mouse, subcutaneous, Uterotrophic Bioassay in Rodents according to OECD 440, non-GLP, with the registered substance, [redacted] 2003 and 2004, rel. 2
- Weight of evidence study: [redacted] (2009) [redacted], non-GLP, with the registered substance, rel. 2.
- Weight of evidence study: [redacted] (2005) [redacted], non-GLP, with the registered substance, rel. 2
- Weight of evidence study: [redacted] (2000) [redacted], non-GLP, with the registered substance, rel. 2.-
- Weight of evidence study: [redacted] (2002) [redacted], non-GLP, with the registered substance, rel. 2
- Weight of evidence study: [redacted] (2007), [redacted]. non-GLP, with the registered substance, rel. 2

ECHA's evaluation and conclusion of the information provided

Evaluation approach

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation while the information from each single source alone is regarded insufficient to support this notion.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance at equivalent level as investigated in an extended one-generation reproductive toxicity study (EU B.56./OECD TG 443) as requested in this decision.

ECHA considers that this study provides, in addition to information to general toxicity, information in particular on two aspects, namely on sexual function and fertility in P0 and F1 generations (further referred to as 'sexual function and fertility') and on development and toxicity of the offspring from birth until adulthood due to pre- and postnatal and adult exposure in the F1 generation and F2 generation until weaning (further referred to as 'effects on offspring').

Relevant elements for 'sexual function and fertility' are in particular functional fertility (oestrous cycle, sperm parameters, mating behaviour, conception, pregnancy, parturition, and lactation) in the P0 and F1 parental generations after sufficient pre-mating exposure and histopathological examinations of reproductive organs in both P and F1 generations. Relevant elements for 'effects on offspring' are in particular peri- and post-natal investigations of the F1 generation up to adulthood including investigations to detect endocrine disruptive properties, investigations on developmental neurotoxicity, postnatal development of F2 generation. Also the sensitivity and depth of investigations to detect effects on 'sexual function and fertility' and 'effects on offspring' needs to be considered.

Furthermore, the relative values/weights of different pieces of the provided information needs to be assessed as indicated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.4., Section 4.4 (version 1.1, December 2011). In particular relevance, reliability and consistency of results/data and coverage (completeness) need to be considered.

Sexual function and fertility

While to the provided studies a Klimisch reliability score of 1 or 2 was assigned in the dossier, ECHA notes that there is not a consistent outcome of these studies as both positive and negative response/evidence were obtained from different studies. Furthermore, not all the key parameters for this endpoint are sufficiently covered with adequate data. While reproductive organs and sperm parameters and some hormone levels have been examined there was no reproductive cycle (cycle including conception and extending through gestation and parturition) in any of the studies. You did not explain why and how the information on various aspects of reproduction provided by an extended one-reproductive toxicity could be replaced or predicted for your substance by histopathological examinations only from other types of studies.

Thus, the information you provided does not support your conclusion that the substance does not have a dangerous property with respect to sexual function and fertility.

Post-natal developmental toxicity

ECHA notes that your adaptation justification does not address the post-natal developmental toxicity. The provided information does also not cover the key elements which need to be investigated in this regard. The studies according to OECD TG 414 in the rat, mouse and hamster, provide information only on pre-natal developmental toxicity but do not cover the peri-and postnatal developmental toxicity. Thus, the information you provided does not support the conclusion that the substance does not have a hazardous property with respect to postnatal developmental toxicity.

Conclusion

Hence, the information you provided to support you adaptation, considered individually or together, does not allow to assume/conclude that the substance does not have a particular dangerous (hazardous) property with respect to the information requirement for Annex IX, Section 8.7.3.

Therefore, the general rules for adaptation laid down in Annex XI, Section 1.2 of the REACH Regulation are not met and your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according to Annex IX, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the required study

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to the ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6.0, July 2017), the starting point for deciding on the length of the premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required if there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6.0, July 2017).

In this specific case, animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be 10 weeks for these Cohort 1B animals and the fertility parameters will be covered allowing an evaluation of the full spectrum of effects on fertility in these animals. Thus, shorter premating exposure duration for parental (P) animals may be considered. However, the premating period shall not be shorter than two weeks and must be sufficiently long to reach a steady-state in reproductive organs as advised in the ECHA Guidance. The consideration should take into account whether the findings from P animals after a longer premating exposure duration would provide important information for interpretation of the findings in F1 animals, e.g. when considering the potential developmental origin of such findings as explained in ECHA guidance.

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main

study. This will support the justifications of the dose level selections and interpretation of the results.

Extension of Cohort 1B

If the column 2 conditions of 8.7.3., Annex IX are met, Cohort 1B must be extended, which means that the F2 generation is produced by mating the Cohort 1B animals. This extension provides information also on the sexual function and fertility of the F1 animals.

The use of the registered substance in the joint submission is leading to significant exposure of consumers and professionals as it is used in cosmetics and personal care products.

Furthermore, the registered substance showed indications for a potential endocrine-disrupting modes of action. In a 56-day repeated dose dietary study in the dossier ([redacted] 2005) with methylparaben (the registered substance subject to this decision) administered *ad libitum* to male rats in doses of 100 ppm, 1000 ppm, 10000 ppm (equivalent to 11.2, 110.0 and 1141.1 mg/kg bw/d), *"The number of normal sperm was significantly reduced (p</=0.05) and corresponding values for percent abnormal were significantly increased in the 1000 ppm (p</=0.01) and 10000 ppm (p</=0.05) exposure groups."*

A female rat study by Vo et al. (2010) found in literature, revealed a "*significant decrease in serum estradiol and thyroxine concentrations in methyl-, ethyl-, propyl-, isopropyl-, and isobutylparaben-treated groups*."

Therefore, ECHA concludes that Cohort 1B must be extended to include mating of the animals and production of the F2 generation because the uses of the registered substance is leading to significant exposure of consumers and professionals and there are indications of endocrine disrupting modes of action.

The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity as described in column 2 of 8.7.3., Annex IX. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

ECHA notes that existing information on the registered substance itself and/or substances structurally analogous to the registered substance derived from available in *vivo* studies (Vo et al. 2010, Ali and Elgoly (2013)[3] show evidence of thyroid toxicity and neurotoxicity.

ECHA guidance (Chapter R.7a: Endpoint specific guidance Version 5.0 – December 2016)(Appendix R.7.6–2, EOGRTS Study Design) specifies that there is a concern for developmental neurotoxicity if:

- There are relevant changes in thyroid hormone levels or signs of thyroid toxicity indicating such changes
- Structurally analogue substances show (developmental) neurotoxic effects in *in vivo* or *in vitro* studies suggesting that similar effects or similar mechanisms/modes of action are likely to apply also for the registered substance.

Vo et al (2010), suggested the effects of parabens, including the registered substance, as thyrotoxic during the prepubertal stage of development in female rats. They observed a significant decrease in serum thyroxine concentrations in methyl-, ethyl-, propyl-, isopropyl, and isobutylparaben-treated groups. In addition, the registered substance caused an increase in thyroid weight.

Ali and Elgoly (2013) have reported neuro-developmental disorders similar to some of the neurodevelopmental disorders observed in the Valproic acid model of autism, following prenatal exposure to butylparaben, a structurally related member of the paraben family.

ECHA concludes that the developmental neurotoxicity cohorts 2A and 2B need to be conducted because there is a particular concern on (developmental) neurotoxicity based on the results from the above-identified studies on the registered substance itself and on substances structurally analogous to the registered substance.

The study design must be justified in the dossier and thus the existence/non-existence of the conditions/triggers must be documented.

Cohort 3

Proposals for amendment (PfAs) submitted by some Member State Competent Authorities (MSCAs) suggested that the developmental immunotoxicity Cohort 3 needs to be included.

In your comments on the PfAs you agreed to conduct the developmental immunotoxicity Cohort 3 "*to ensure comparability in the respective testing design of the OECD TG 443 with the 'extended one generation study' which was simultaneously required by ECHA also for propyl 4-hydroxybenzoate*" and also for the purpose of providing information for a read-

[3] **Ali EH, Elgoly AH 2013.** Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: comparison with valproic acid autistic model. Pharmacol Biochem Behav. 2013 Oct;111:102-10.

across justification for another substance.

According to Article 1(3) of the REACH Regulation, you need to ensure that the manufacture, placing on the market or the use of the substance does not adversely affect human health. In line with Annex I, Section 0.5, of the REACH Regulation, in your comments you have considered and explained why the additional information (cohort 3) is necessary. Accordingly, the developmental immunotoxicity Cohort 3 is included in the requested study design.

The study design must be justified in the dossier.

Species and route selection

According to the test method EU B.56/ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a water soluble solid, ECHA concludes that testing should be performed by the oral route.

c) Outcome

Based on the available information, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- At least two weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation;
- Cohorts 2A and 2B (Developmental neurotoxicity); and
- Cohort 3 (Developmental immunotoxicity).

Appendix 2: Procedural history

ECHA notes that the tonnage band for one member of the joint submission is more than 1 000 tonnes or more per year.

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 14 November 2016.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the requests.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposals for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendments).

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

In addition, you provided comments on the draft decision. These comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-59 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.