Helsinki, 6 September 2016

Decision number/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)

DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For methyl 4-hydroxybenzoate, CAS No 99-76-3 (EC No 202-785-7)

Addressees: Registrant(s)[1] of methyl 4-hydroxybenzoate

This decision is addressed to the Registrant(s) of the above substance with active registrations pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided as an Annex to this decision.

Based on an evaluation by Anses as the French Competent Authority (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 4 May 2015, i.e. the day on which the draft decision was notified to the Registrant(s) pursuant to Article 50(1) of the REACH Regulation.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the French Competent Authority has initiated substance evaluation for methyl 4-hydroxybenzoate, CAS No 99-76-3 (EC No 202-785-7) based on registrations submitted by the Registrants and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

1 The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected CMR, wide dispersive use, consumer use, exposure of sensitive population, high aggregated tonnage and suspected endocrine disruptor, methyl 4-hydroxybenzoate was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The updated CoRAP was published on the ECHA website on 26 March 2014. The Competent Authority of France was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA identified skin sensitization and skin irritation as additional concerns regarding human health.

The evaluating MSCA considered that further information was required to clarify the following concerns: suspected skin irritant, suspected skin sensitizer, suspected CMR, suspected endocrine disruptor. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 25 March 2015.

On 4 May 2015 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant(s) commenting phase

By 10 June 2015 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA.

The evaluating MSCA considered the comments received from the Registrant(s). On the basis of this information section II was amended. The statement of reasons (section III) was changed accordingly.

The initial requests for an extended one-generation reproductive toxicity study, a skin sensitisation study and a skin irritation study were removed from this decision as they were mainly based on concerns raised by missing REACH standard information.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 3 March 2016 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, one Competent Authorities of the Member States and ECHA submitted proposals for amendment to the draft decision.

On 8 April 2016 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment received and amended the draft decision.

Referral to Member State Committee

On 18 April 2016 ECHA referred the draft decision to the Member State Committee.

By 10 May 2016, the Registrant(s) provided comments on the proposals for amendment, in accordance to Article 51(5) and on the draft decision. The Member State Committee took your comments into account and they are reflected in Section III.

After discussion in the Member State Committee meeting on 6-10 June 2016, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 6 June 2016. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods (in accordance with Article 13(3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

> Fish Sexual Development Test (FSDT, test method: OECD 234) with Japanese medaka *Oryzias latipes* or *Zebrafish Danio rerio*. The genetic sex determination and secondary sex characteristics shall also be included if the determination of the parameters is possible for the selected test species.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **13 March 2018** an update of the registration(s) containing the information required by this decision[2], including robust study summaries and, where relevant, an update of the Chemical Safety Report. For a better assessment of the reliability of this study, in addition to the robust study summary provided in the registration dossier, the full study report must be provided, in order to have access to the raw data.

III. Statement of reasons

Based on the evaluation of all relevant information submitted on methyl 4-hydroxybenzoate and other relevant and available information and taking into account the comments of the Registrant(s) concerned, proposals for amendment submitted by Member State Competent Authorities/ECHA and the deliberations of the Member State Committee, ECHA concludes that further information is required in order to enable the evaluating MSCA to complete the evaluation of whether the substance constitutes a risk to human health or the environment.

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

Environment concerns

Fish Sexual Development Test (FSDT, OECD TG 234)

Methyl 4-hydroxybenzoate was included in the CoRAP in particular because of its potential endocrine disrupting (ED) properties. No data on these potential ED properties for the environment was provided in the registration dossier, although *in vivo* data on fish for this type of effects were available in the open literature, as detailed here below.

Barse *et al.* (2010; RI=2)[3] exposed male of *Cyprinus carpio* during 28 days to methyl 4-hydroxybenzoate at nominal concentration of 0.84, 1.68, and 4.2 mg/L. The measured endpoints (vitellogenin, enzymatic activities, gonado- and hepato-somatic index, liver and gonad histology) permitted to conclude that methyl 4-hydroxybenzoate has a potential estrogenic activity. This conclusion is mainly supported by the significant induction of vitellogenin in males for all tested concentrations, following a non-monotonic response, as the highest induction was measured at the lowest tested concentration. In addition, gonad histology indicated a potential adverse effect on spermatogenesis, as the authors observed a reduced number of spermatozoa in testis of males exposed to methyl 4-hydroxybenzoate.

Yamamoto *et al.* (2011; RI=3)[4] exposed male adults of *Oryzias latipes* during 14 days to several parabens, including methyl 4-hydroxybenzoate at the measured concentrations of 10, 160, 780, 4500, and 24000 µg/L. For methyl 4-hydroxybenzoate, the measurement of vitellogenin concentrations showed a significant induction with a dose response relationship for tested concentrations above 160 µg/L. This result indicates that methyl 4-hydroxybenzoate has a potential estrogenic activity in fish.

Both studies support the hypothesis of an estrogenic activity in fish for methyl 4-hydroxybenzoate. This is in accordance with *in vitro* data that revealed a significant affinity of methyl 4-hydroxybenzoate for estrogenic receptors (Blair *et al*., 2000[5] and Byford, 2002[6]). Nevertheless, more robust data are needed to confirm this hypothesis of an estrogenic activity in fish.

The two published studies described above should be considered at level 3 of the conceptual framework (*i.e. in vivo* assays providing data about selected endocrine mechanism(s) / pathway(s)) according to the OECD Guidance document on standardized test guidelines for evaluating chemicals for endocrine disruption (OECD, 2012a)[7]. Their results (*i.e.* mainly the significant vitellogenin induction in fish male adults) indicate possibilities for adverse effects, which can be highlighted in reproductive and developmental studies of levels 4 and 5. According to the OECD guideline, studies of level 4 or 5 that highlight adverse effects linked to the mode of action are needed to identify endocrine disruptive substance.

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[3] Barse V., T. Chakrabarti, T. K. Ghosh, A. K. Pal, N. Kumar, R. P. Raman, and S. B. Jadhao (2010). Vitellogenin induction and histo-metabolic changes following exposure of *Cyprinus carpio* to methyl paraben. Asian-Australasian Journal of Animal Sciences 23 (12):1557-1565.

[4] Yamamoto, H., Tamura, I., Hirata, Y., Kato, J., Kagota, K., Katsuki, S., Yamamoto, A., Kagami, Y., and Tatarazako, N. (2011). Aquatic toxicity and ecological risk assessment of seven parabens: Individual and additive approach. Science of The Total Environment 410-411, 102-111.

[5] Blair, R. M. et al. (2000). The Estrogen Receptor relative Binding Affinities of 188 Natural and Xenochemicals: Structural Diversity of Ligands. Toxicological Sciences 54, 138-153.

[6] Byford, J. R. et al. (2002). Oestrogenic Activity of Parabens in MCF7 Human Breast Cancer Cells. Journal of Steroid Biochemistry and Molecular Biology 80, 49-60.

[7] OECD, (2012a). Guidance document n°150 on standardized test guidelines for evaluating chemicals for endocrine disruption. ENV/JM/MONO(2012)22.

Additionally the concern for possible ED effects in wildlife is also raised by the effects seen on the thyroid hormone system (reduced thyroid hormones levels and increased thyroid weight in peripubertal female rats were observed) in the study by Vo *et al.*, (2010)[8].

The Fish sexual Development Test (FSDT; OECD TG 234) is a partial lifecycle assay that can be used to show several types of *in vivo* endocrine disruption activities in fish, including estrogenic activity, and also to provide apical information relevant for the environmental risk assessment. This test is recommended by OECD (2012) as a conceptual framework level 4 test that covers a sensitive fish life stage responsive to both estrogen and androgen-like chemicals. Performing this test should allow confirming if methyl 4-hydroxybenzoate has an estrogenic activity on fish, and if this estrogenic activity induces adverse effect on fish sexual development.

During the commenting period, the Registrant(s) made several comments on the low reliability of the two available *in vivo* studies on fish[3,4]. To summarize, the Registrant(s) considers that both studies have significant shortcomings and the scientific value of the presented data to postulate endocrine activity is severely limited. According to the OECD 'Fish Toxicity Testing Framework Document' (OECD, 2012b)[9] one of the key factors when deciding on testing requirements for endocrine properties in aquatic environments is the strength of the evidence indicating such properties. Based hereupon and taking into account that — if at all — the presented evidence on the potential endocrine property of methyl 4-hydroxybenzoate is not very strong, the requirement of an OECD 234 'Fish Sexual Developement Test' in their opinion is unjustified.

ECHA agrees with the Registrant(s) that the reliability of both studies is insufficient – mostly due to insufficient description of the applied protocol and results – for drawing a conclusion on the ED properties of the methyl 4-hydroxybenzoate. Nonetheless their results bring keypoints on the potential estrogenic activity of the methyl 4-hydroxybenzoate (*i.e.* VTG concentration), which reasonably justify the request for more robust data on the endocrine activity of the methyl 4-hydrobenzoate. Indeed, the main result of both studies that should be considered is the significant VTG induction in fish male exposed to methyl 4-hydroxybenzoate.

Concerning the study of Barse *et al.* (2010), the Registrant(s) pointed out the non common methodology used by the authors for the vitellogenin determination, which was measured in muscle sample. According to the Registrant(s), blood or liver are generally used to measure vitellogenin induction but not fish muscle tissue. However, a scientific publication indicated that vitellogenin is potentially present in most of fish tissues (Zhong *et al.*,2014[10]).

[8] Vo *et al.* (2010) Potential estrogenic effect(s) of parabens at the 21 prepubertal stage of a postnatal female rat model. Reproductive Toxicology 29:306-316.
[9] OECD (2012b). Fish toxicity testing framework. Series on testing and assessment No. 171. ENV/JM/MONO (2012)16
[10] Zhong et al. (2014). Distribution of vitellogenin in zebrafish (*Danio rerio*) tissues for biomarker analysis. Aquatic Toxicology 149, p. 1-7.

This publication on zebrafish demonstrated that exposure of males to an estrogenic substance is linked to a VTG induction in most of the tissues, but with different magnitude. Considering that zebrafish and carp are both oviparous species, and vitellogenin is the egg yolk precursor protein, the same distribution could be expected for both species.

The Registrant(s) also highlight that although generally vitellogenin induction results in enhanced liver metabolism leading to an enlargement of the liver and consequently an increased hepatosomatic index, the results however show decreasing vitellogenin induction with increasing hepatosomatic index (HSI). In addition, with regard to enzyme activities significant differences among treatment groups with no clear dose-response were observed. ECHA is of the opinion that this non-expected relation between VTG induction and HSI increase, and the non clear dose-response relationship for most of measured endpoints could be explained by the hepatotoxicity of the methyl 4-hydroxybenzoate occurring in treated fishes at the higher tested concentrations (1.68 and 4.2 mg/L). The hypothesis of an hepatotoxicity of the methyl 4-hydroxybenzoate is supported by the histology of the liver from treated fish, where a general increase in vacuoles and appearance of focal necrosis of hepatocytes were observed compared to control. As mentioned in the OECD conceptual framework, hepatotoxicity could involve that VTG induction is masked, and hence explain why no clear dose-response relationship for the VTG induction might be seen in treated male fishes.

Nonetheless, it should be noted that for all tested concentrations, the VTG induction in males was significant compared to control, which indicates a potential estrogenic activity, keeping in mind the absence of dose-response relationship, probably because of a too elevated tested concentration range.

Concerning the study of Yamamoto *et al.* (2011), the Registrant(s) point out for VTG concentration a high inter-study variability in negative control (*i.e.* one study for one tested paraben with its own concentration range including negative control), with concentration up to 10000 ng/mL. Such level and variability between studies in control for VTG concentrations in male plasma have already been observed during the validation process of the actual OECD TG 229 and 234 (*c.f.* references in both OECD technical guidances for more details). It should be noted that for OECD TG on fish with VTG measurements, no validity criteria is mentioned about VTG level and variability in negative control. As a consequence, it is questionable to invalidate any study based on the criteria, when OECD TGs do not.

As commented by the Registrant(s), and in accordance with the ECHA evaluation conclusion, the *in vivo* tests on fish should be considered as supportive data considering their low reliability. In a weight of evidence approach, those studies support the potential estrogenic activity of the methyl 4-hydroxybenzoate. Anyhow, as adverse effect should be linked to the endocrine mode of action for considering a substance as an endocrine disruptor, additional data are needed to confirm for methyl 4-hydroxybenzoate the hypothesis of an estrogenic activity and its potential associated adverse effect on fish.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision:

Fish Sexual Development (Test OECD TG 234) with Japanese medaka *Oryzias latipes* or *Zebrafish Danio rerio*. The genetic sex determination and secondary sex characteristics shall also be included if the determination of the parameters is possible for the selected test species.

Five test concentrations must be tested in a range between 0.1 and 10 mg/L expressed in measured concentration, in order to cover the highest tested concentration recommended by the OECD 234 standard guideline and the concentration for which significant effects were demonstrated in the studies of Barse *et al.* (2010) and Yamamoto *et al.* (2011).

For a better assessment of the reliability of this study, in addition to the robust study summary provided in the registration dossier, the full study report must be provided, in order to have access to the raw data.

During the commenting period, the Registrant(s) agreed with ECHA that new data is needed for assessing the endocrine disrupting properties of the methyl 4-hydroxybenzoate by proposing a tiered testing strategy.

The Registrant(s) proposed as a first tier to clarify the presumed estrogenic activity of methyl 4-hydroxybenzoate using appropriate and validated in vitro assays based on approved OECD test guidelines and ensuring Good Laboratory Practice (*e.g.* OECD 455, OECD 457 or PBTG on human recombinant estrogen receptor alpha binding assay according to existing OECD Draft Guideline). In case of positive results in the first tier, the Registrant(s) proposed in a second tier to investigate whether effects may also occur in vivo by running an OECD TG 229. Only in case of remaining reasonable doubts further higher tier testing according to an OECD 234 'Fish Sexual Development Test' should be considered.
ECHA is of the opinion that available data are sufficient for clearly demonstrate the potential estrogenic activity of methyl 4-hydroxybenzoate. By applying a weight of evidence approach, available data support the hypothesis of an estrogenic activity as mode of action for methyl 4-hydroxybenzoate in fish. *In vitro* data revealed a significant affinity of methyl 4-hydroxybenzoate for estrogenic receptors (Blair *et al*., 2000 and Byford, 2002). *In vivo* tests on fish (level 3 of the conceptual framework) indicated a significant vitellogenin induction in fish male exposed to methyl 4-hydroxybenzoate.

Additionally one Competent Authority in its proposal for amendment clearly supported the information requirement, i.e. the level 4 FSDT (OECD 234). Therefore the Registrant(s) comment on the proposal for amendment suggesting to take a tiered approach starting with level 2 studies cannot be considered at this stage.

As a consequence, new data on the mode of action of methyl 4-hydroxybenzoate (level 2 of the conceptual framework) is not needed. A higher level test according to the conceptual framework should be directly performed in order to confirm for methyl 4-hydroxybenzoate the hypothesis of an estrogenic activity and its potential associated-adverse effect on fish.

To conclude, ECHA takes into account the Registrant(s) tier approach proposal, but considers the initial request more appropriate and proportional, considering the available data on the estrogenic activity of the methyl 4-hydroxybenzoate, and the possibility to clearly conclude on the estrogenic activity associated with adverse effect with just one additional new test, in case of positive results for vitellogenin and sex ratio endpoints in the OECD TG 234.

In their comments on the proposals for amendment, the Registrant(s) indicated that they would prefer the zebrafish as the test organism to be used for the FSDT (TG: OECD 234).

IV. Adequate identification of the composition of the tested material

In relation to the required experimental studies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrants. It is the responsibility of all the Registrants to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the test(s) must be shared by the Registrant(s).

V. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrants (Article 53 of the REACH Regulation). Registrants are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:

https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at
https://echa.europa.eu/regulations/reach/registration/data-sharing.

If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.

In the original draft decision the time indicated to provide the requested information was 24 months from the date of adoption of the decision. This period of time took into account the fact that the draft decision also requested an extended one-generation reproductive toxicity study. As these requests are not addressed in the present decision, ECHA considers that a reasonable time period for providing the currently required information in the form of an updated registration is 18 months from the date of the adoption of the decision. The decision was therefore modified accordingly.

VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised by Leena Ylä-Mononen[11], Director of Evaluation

Annex: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

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