Ministry of Environment and Food of Denmark
Environmental
Protection Agency

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Benzophenone
EC No 204-337-6
CAS No 119-61-9

Evaluating Member State(s):   Denmark

Dated: 05. April 2018

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation a Decision to request further information was issued on 30 November 2015. After having reviewed the submitted information the Member State concluded the evaluation without any further need to ask more information from the Registrant(s) under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the Registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

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[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Benzophenone was originally selected for substance evaluation in order to clarify concerns about:

- carcinogenicity

- wide dispersive use, consumer use and a high risk characterisation ratio (RCR)

During the evaluation also another concern was identified. The additional concern was:

- endocrine disrupting effects

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

The European Food Safety Authority (EFSA), has in a Scientific Opinion from 2009 re-assesed the TDI on Benzophenone and its metabolite, hydroxybenzophenone, based on new toxicological studies available in 2009 (EFSA, 2009). On this basis, EFSA set a TDI of 0.03 mg/kg bw/day. Benzophenone is included in Annex 1 of Regulation 10/2011 on food contact materials with a specific migration limit of 0.6 mg/kg food.

In a Scientific Opinion from EFSA dated 20. September 2017 the safety of Benzophenone used as flavouring was assessed. Overall the EFSA Panel concluded that the TDI of 0.03 mg/kg bw/day for Benzophenone is appropriate to cover the non-neoplastic effects in the chronic toxicity studies and the neoplastic effects included in the rodent carcinogenicity studies, and that there is no safety concern for Benzophenone under the current condition of use as a flavouring substance (EFSA 2017).

Benzophenone is listed in the 1st Update of the Inventory of Ingredients Employed in Cosmetic Products Section II: Perfume and Aromatic Raw Materials (2000). Benzophenone is not listed in Annex VI to Cosmetic Regulation No 1223/2009, which is the list of UV-filters allowed in cosmetic products, and Benzophenone must therefore not be used as a UV-filter in cosmetic products.

Benzophenone is listed with the functions "masking" and "UV absorber" in the CosIng database from the European Commission.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	

Restrictions	
Other EU-wide measures	
Currently no regulatory follow-up action at EU level	

3.1. Carcinogenicity

In the justification for CoRAP, the initial concern for carcinogenicity was based on results of carcinogenicity studies in mouse and rats where tumours were observed. In addition, a genotoxicity test (SOS/umu test), conducted with human P450s was reported to be positive.

The evaluation of the available data on the mutagenicity of Benzophenone, which consisted of negative *in vitro* OECD guideline studies (in bacteria, one mammalian cell gene mutation assay, one unscheduled DNA synthesis assay) and the above mentioned non-guideline SOS/umu gene expression test led to the overall conclusion that Benzophenone is not genotoxic.

No information on carcinogenicity in humans is available. Benzophenone has been tested in animals in two different species and included a 120 week study with dermal exposure from 1974 in mice and a 105-week study reported by NTP in 2006 in mice and rats. The studies are outlined in part B of the present document.

Tumour data from the study from 1974 do not indicate a potential carcinogenicity of Benzophenone in mice following a topical administration under conditions of this bioassay. It is noted by the evaluating MS that the dermal study from 1974 was not performed according to Good Laboratory Practice (GLP) and Organisation for Economic Co-operation and Development (OECD) guidelines. However the study is included in the evaluation as a part of supportive evidence on the possible carcinogenic effects.

Concerning the 105 week study the evaluating MS concluded that the available long-term studies in mice and rats performed by NTP according to Good Laboratory Practice (GLP) and Organisation for Economic Co-operation and Development (OECD) guidelines are appropriate for evaluating the potential carcinogenicity of Benzophenone.

Benzophenone exerts tumourigenic effects in rats and mice in the liver, the kidney and in the haematopoetic system, including rare histiocytic sarcomas. Incidences of neoplasms differ between species and sexes. As the available evidence supports that Benzophenone is not genotoxic, a threshold for its carcinogenic effect may be identified.

Overall, based on the available evidence, the evaluating MS concludes that Benzophenone meets the criteria for classification as carcinogenic in category 2. A proposal for harmonised classification for carcinogenic effect is thus needed, as required by CLP article 36 (c).

No additional long-term studies are required as a conclusion to the substance evaluation of Benzophenone, as such studies are not expected to yield substantial further relevant information for the classification of Benzophenone as carcinogenic.

3.2. Exposure and risk

The initial concern related to the possible risk to humans from exposure to Benzophenone, resulted in a request in a decision dated 30 November 2015 to the Registrant(s) for more detailed information on the human exposure scenarios and/or a possible introduction of further Risk Management Measures (RMMs). The Registrant(s) provided supplementary data on

exposure during the evaluation process. The provided data were sufficient for the evaluating MS to withdraw the initial requests related to the concerns about human exposure. The evaluating MS therefore concludes that no further action is needed on these end-points.

3.3. Endocrine disrupting properties

3.3.1. Estrogenic potential

During the substance evaluation process, a concern arose regarding potential endocrine disruptive effects of Benzophenone and its metabolites/degradation products (estrogenic potential and thyroid disruption). The concern is raised by estrogenic activity of Benzophenone metabolites and environmental degradation products *in vitro* and *in vivo* (in the uterotrophic assay).

Based on the performed evaluation, the evaluating MS considers that currently there is no straightforward way to follow up on the concern for human health in respect to the oestrogenic activity of Benzophenone, since a number of long-term rodent studies are already available, including an older 2-generation reproductive toxicity study, which did not detect effects in some endpoints sensitive to endocrine disruption (i.e. anogenital distance F1 and F2 offspring, timing of sexual maturation in F1 offspring, weights and histopathological evaluation of testes, epididymis, prostates, seminal vesicles, ovaries and uterus in F1 parental animals, and levels of testosterone, FSH, LH and estradiol, estrous cyclicity and semen quality in F0 and F1 animals). It is acknowledged that the most sensitive endpoints currently known for detection of estrogenic effects were not included in this 2-generation study (e.g. evaluation of mammary gland whole mounts in young female offspring and quantitative assessment of follicular maturation in adult female offspring). However, even though the doses used in the 2-generation study may have been too low to induce estrogenic effects (when compared to the doses inducing estrogenic effects in the uterotrophic assays), the highest dose induced some systemic toxicity, and testing in higher doses is therefore evaluated not to be appropriate in a similar test design. Further, currently no reliable short term test methods exist which could be requested for a more thorough, proportionate and adequate investigation of whether the estrogenic mode of action of Benzophenone might lead to adverse effects in rodents.

Concerning the environment, following up on the concern for estrogenic potential is not straightforward either. Based on the considerations about the environmental fate properties of Benzophenone and its environmental transformation products (c.f. section 7.7), the evaluating MS finds it very uncertain, if further efforts to clarify the estrogenic potential of the metabolites/degradation products of Benzophenone in the context of substance evaluation under REACH would lead to improved risk management of the substance. Further testing would include several steps, possibly first targeting the environmental degradation products (concerning their chemical identity, their quantitative formation and their further biodegradation rates), followed by endocrine targeted testing of any potentially relevant degradation products known to have estrogenic activity. In addition, there would be a high uncertainty as to whether this might lead to a regulatory relevant outcome in respect to identification of Benzophenone as a SVHC in accordance with REACH art. 57 (f). First of all it is unknown whether relevant degradation products/metabolites such as for example 4-hydroxy Benzophenone would cause adverse effects in fish due to the estrogenic activity, but apart from identification of the substance as an endocrine disruptor in the environment, an SVHC identification also requires an assessment of whether the substance causes equivalent concerns as CMR- or vPvB/PBT substances. Based on the available information for Benzophenone regarding the indication for endocrine disruptive effects and environmental fate properties it is considered very uncertain if such a decision would be reached.

In conclusion, the evaluating MS currently does not consider it to be proportionate to request further testing in line with the testing strategy outlined above, which would be required to fully

clarify the remaining indications of potential endocrine properties with respect to estrogenic effects on wildlife.

Therefore, for the time being, the evaluating MS does not consider this substance to be of priority for further action under substance evaluation with respect to endocrine disruptive properties.

3.3.2. Thyroid disruptive potential

For thyroid disruptive potential, the initial concern was raised by inhibition of TPO activity *in vitro*.

With regards to assessment of the concern for human health, the most sensitive parameter for thyroid disruption (i.e. thyroid hormone levels) was not measured in any of the available *in vivo* rodent studies, leaving some residual concern for thyroid toxicity. However, a large number of long term studies in rodents are available, and thyroid histology and pathology were not affected in any of the studies, including in 2-years carcinogenicity studies in mice and rats. Thus, the overall weight of evidence analysis indicates that Benzophenone is not likely to be a thyroid disrupting substance in rodents *in vivo*.

There is, however, some residual concern that Benzophenone could induce thyroid toxicity in aquatic wildlife such as amphibians. However, the degree of concern is low due to the fast transformation of Benzophenone and its low bioaccumulation potential (see section on environmental fate, 7.7). Further testing on this readily biodegradable substance with low bioaccumulation potential would first require an AMA for investigating whether Benzophenone has thyroid activity in amphibians, and if so to make a follow-up request for a LAGDA test to investigate if such thyroid activity might led to adverse developmental effects in amphibians.

The degree of concern is low. As indicated above it is based on available information unlikely that Benzophenone causes adverse effects due to interference with the thyroid system in rodents. Furthermore, it should also be taken into account that Benzophenone undergoes quite fast transformation and that Benzophenone has a low bioaccumulation potential. Therefore, in this specific case, the evaluating MS currently considers it to be too uncertain whether further testing would lead to improved risk management.

Therefore for the time being the evaluating MS does not consider this substance to be of priority for further action under substance evaluation with respect to endocrine disruption properties.

The evaluating MS notes that a classification as carcinogen in category 2 is proposed. Such a classification will entail additional risk management measures leading to a reduced risk – also from the potential effects on endocrine disruption.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

The data available in the registration dossier are deemed sufficient to warrant a classification as carcinogenic in category 2. However, the self-classification notified by the Registrant(s) does not include this classification. According to article 36 (c) of regulation 1907/2008 (Classification, Labelling and Packaging, (CLP)) classification for carcinogenic effects should be harmonised. Therefore, a classification proposal for Benzophenone for carcinogenic effects should be prepared.

4.1.1. Harmonised Classification and Labelling

Benzophenone is not at present included in Annex VI with a harmonised classification. Thus, the proposal will be a new entry in CLP-Annex VI.

As referred above under point 3 "conclusion on substance evaluation" the evidence available shows that Benzophenone is tumourigenic in animals in several organs. The data are deemed sufficient to warrant a classification as carcinogenic in category 2.

A classification as carcinogenic would lead to risk management through e.g. the cosmetics directive and possibly also the working environment directives and in other down-stream regulations and voluntary reduced marketing and use of the substance. These initiatives are likely to increase protection of workers and consumers from exposure to the substance.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

No other actions other than a harmonised classification are considered at this point in time.

4.1.3. Restriction

No other actions other than a harmonised classification are considered at this point in time.

4.1.4. Other EU-wide regulatory risk management measures

No actions other than a harmonised classification are considered at this point in time.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
CLP Annex VI dossier for harmonised classification (CLP) for carcinogenic effect	December 2018	Member State: Denmark

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Benzophenone was originally selected for substance evaluation in order to clarify concerns about:

- carcinogenicity

- wide dispersive use, consumer use and a high risk characterisation ratio (RCR)

During the evaluation also other concern was identified. The additional concern was:

- endocrine disrupting effects

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Carcinogenicity	Carcinogenicity confirmed (category 2). Not genotoxic in laboratory animals. Additional long-term studies are not expected to yield further relevant information that could change present hazard identification and risk characterization. Harmonised C&L process to be initiated.
Wide dispersive use, consumer use and a high risk characterisation ratio (RCR)	Additional data on exposure showed no risk at wide dispersive use and consumer use and that the current risk characterisation ratio (RCR) is below 1. There are sufficient data on exposure. No need for further action.
Endocrine disrupting effects	There is some residual indications for potential endocrine disruption (estrogenic and thyroid disrupting effects). However, the evaluating MS considers it to be very uncertain, if requests for further testing would result in improved risk management. No further action.

7.2. Procedure

Benzophenone was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013 due to initial grounds for concern relating to carcinogenicity, wide dispersive use, consumer use and a high risk characterisation ratio (RCR).

On 20 March 2013 the updated CoRAP was published on the ECHA website and the Competent Authority of Denmark was appointed to carry out the evaluation.

On 17 March 2014 the evaluating Member State (evaluating MS) submitted a draft decision to ECHA. In the course of the evaluation, the evaluating MS concluded that it would not be necessary to propose further tests on carcinogenicity in order to clarify the identified concern, but noted additional concerns regarding endocrine disrupting effects. The evaluating MS considered that further information was required to clarify the concern on endocrine disrupting effects. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information.

On 29 April 2014 ECHA sent the draft decision to the Registrant(s) and invited them to provide comments.

By 3 June 2014 ECHA received comments from the Registrant(s). The evaluating MS considered the comments from the Registrant(s) and the dossier update, and on basis of this information, Section II in the draft decision was amended and Section III was changed accordingly.

On 23 July 2015 the evaluating MS notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them to submit proposals to amend the draft decision. Subsequently, some Competent Authorities of the Member States and ECHA submitted proposals for amendment to the draft decision.

On 28 August 2015 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them to provide comments on those, and referred the draft decision to the Member State Committee on 7 September 2015.

On 28 September 2015 the Registrant(s) provided comments on the proposals for amendment.

On 27-29 October 2015 the discussion in the Member State Committee meeting resulted in a change of the information requested in the draft decision. A unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 29 October 2015.

On 30 November 2015 a final decision was issued by ECHA. The information required was provided by the Registrant(s) before the time limit on 7 June 2016.

On 6 June 2016 the Registrant(s) provided further information on the fate of Benzophenone in the environment, based on his updated literature search on the fate of Benzophenone, performed in 2015 and 2016. Further an updated version of the Chemicals Safety Report were available. The evaluating MS initiated the assessment of the information received.

From March-June 2017 the evaluating MS assessed Benzophenone and its degradation products to be readily biodegradable based on the available literature, and initiated the preparation of the conclusion document for Benzophenone.

On 2 June 2017 the evaluating MS submitted the conclusion document to ECHA. Subsequently some changes to the conclusion were necessary, resulting in this later revision of the document.

7.2.1. Carcinogenicity

Evaluation of existing information on carcinogenicity, March 2013 – March 2014:

A targeted review of studies investigating a potential carcinogenic potential of Benzophenone was undertaken by the evaluating MS with the aim to evaluate whether the reported pre-neoplastic and neoplastic lesions could indicate a carcinogenic effect of Benzophenone. A

review of available *in vitro* and *in vivo* genotoxicity studies with Benzophenone was undertaken in order to evaluate whether a conclusion could be sustained from previous evaluations stating that there was no concern with respect to genotoxicity of Benzophenone. The reviewed papers included available studies from an updated literature search and those which were already present in the REACH resgistration dossier from the Registrant(s). Based on this the evaluating MS concluded that the available studies demonstrated that Benzophenone is tumourigenic in several organs through a threshold mode of action. Also, the evaluating MS concluded that it would not be necessary to propose further tests on carcinogenicity in order to clarify the identified concern as the available studies were robust.

Therefore, no requirements regarding this end-point were included in the decision of 30 November 2015.

7.2.2. Wide dispersive use, consumer use and a high risk characterisation ratio (RCR)

Evaluation of existing information on exposure etc., March 2013 – March 2014:

Examination by the evaluating MS on the exposure scenarios of Benzophenone resulted in requests of further information in order to clarify the concern on exposure risks from wide dispersive use, consumer use as well as a high risk characterisation ratio (RCR). In the final decision of 30 November 2015. The requirements were the following:

a) More detailed information on the use of the substance;
b) More information on personal protective equipment
c) Documentation that risks to workers and consumers are adequately controlled for all exposure scenarios
d) A more detailed description on how the Registrant(s) have estimated combined exposure (combined for all relevant emission/release cources/exposure routes).

The evaluating MS found among others that the LOAEL on 15 mg/kg bw/day for Benzophenone as a basis to deriving a DNEL is divergent from the LOAEL recommended by the EFSAs Scientific Opinion, Toxicological evaluation of Benzophenone. For the purpose of providing urgent advice to risk managers, the EFSA in its statement considered the liver hypertrophy seen in the two-generation reproductive toxicity study (Hoshino *et al.*, 2005) with Benzophenone as adverse effects. Thus, EFSA derived a LOAEL of 6 mg Benzophenone/kg bw/day from this study.

Deviation from assessment under EU legislation shall be justified (REACH Annex I, 0.5). Normally, the study or studies giving rise to the highest concern shall be used to establish the DNEL(s) if there are several studies that address the same effect. If the study or studies giving rise to the highest concern are not used, then this shall be fully justified and included as part of the technical dossier, not only for the study being used but also for all studies demonstrating a higher concern than the study being used.

On 29 April 2014 ECHA therefore sent the draft decision to the Registrant(s) requesting the Registrant(s) to fully justify the use of a LOAEL of 15 mg/kg bw/day as the basis for derivation of the DNEL and submit this information.

In June 2014 the Registrant(s) stated in his comments to the draft decision that a LOAEL of 15 mg/kg bw/day derived from a 2-year carcinogenicity study has been used as basis, which can be seen as a cautious approach, and that the use of the LOAEL of ca. 6 mg/kg bw/day derived from a two-generation reproductive toxicity study may have some disadvantages, as the liver hypertrophy seen in rats was not correlated with an increase in liver weights and may therefore be an adaptive but not a true adverse response. The evaluating MS and ECHA agreed to this and amended the Decision accordingly.

However, based on the examination by the evaluating MS the Registrant(s) were also requested to update the Chemicals Safety Report (CSR) with the information regarding Benzophenone as stated in both the draft decision dated 29 April 2014 as in the final decision of 30 November 2015 as outlined below.

7.2.2.1. More detailed information on the use of the substance

The use of Benzophenone was described very sparsely in the registration dossier. The evaluating MS found that other uses than those described in the CSR should be considered in developing the relevant exposure scenarios, keeping in mind that Benzophenone is also used for several industrial uses and it seems likely that there is a potential for Benzophenone exposure of both professionals and consumers.

The evaluating MS thus concluded, that the provided information was not sufficient to be able to conclude whether all relevant exposure scenarios for all relevant groups of populations and all relevant environmental compartments have been considered and therefore required more details in the registration dossier.

7.2.2.2. More information on personal protective equipment

Personal protective equipment (PPE) like e.g. gloves and respiratory protection, is recommended in the technical dossier. The evaluating MS found that no details were provided regarding PPEs (e.g. type of material, thickness and breakthrough times of the gloves). The evaluating MS therefore requested information on type of gloves and type of respiratory protection where relevant, also taking into account breakthrough times for gloves and clothing and type of filter for the specified respiratory protective equipment.

7.2.2.3. Documentation that risks to workers and consumers are adequately controlled for all exposure scenarios

The evaluating MS had identified high risk characterization ratios (RCRs) related to exposure via the inhalation and dermal routes. In many of the scenarios/contributing scenarios RCR has been estimated to be 1 and close to 1 (>0.91 - 1). High RCRs indicate a need for clarification, and the evaluating MS therefore requested the RCRs to be reconsidered by refining the exposure estimations with the use of e.g. higher tier exposure assessment tools, and further information on RMMs (Risk Management Measures).

7.2.2.4. More detailed description on estimation of combined routes (combined for all relevant emission/release sources/exposure routes)

The evaluating MS had concluded that a combined scenario for all exposure routes is relevant for Benzophenone and therefore requested a combined exposure and risk assessment scenario or at least a convincing justification for stating that combined scenarios for all exposure routes are: "Not relevant" (i.e. in that case to submit the relevant documentation for the adequacy of this statement).

7.2.2.5. Conclusion on Evaluation of existing information on exposure etc.

The Registrant(s) expressed their consent to provide the requested information as outlined above, and has in the CSR of 04/05/2016 updated the information. No further requests are therefore necessary with respect to exposure assessment.

7.2.3. Endocrine disrupting effects

Evaluation of information on endocrine disrupting effects, March 2013 – June 2017:

Available data in the open literature revealed that Benzophenone and some of its metabolites may have endocrine disruptive properties, and an examination of potential endocrine disrupting effect of Benzophenone was included in the evaluation.

A recent *in vitro* study has shown that Benzophenone decreases the activity of the enzyme thyroid peroxidase (TPO), which is essential in thyroid hormone synthesis. Benzophenone could therefore potentially be a thyroid hormone disrupting substance. Unfortunately, none of the performed *in vivo* studies included measurements of thyroid hormone levels. Such data could have efficiently clarified whether the rodent thyroid system is affected by Benzophenone *in vivo,* because altered thyroxine levels are often the most sensitive measure of thyroid axis pertubations. However, since no adverse effects on thyroid gland weight or histopathology (including no adverse effects on thyroid tumor formation) have been seen in any of the performed subchronic and chronic repeated dose toxicity studies, the overall weight of evidence analysis indicates that Benzophenone is not a thyroid disrupting compound *in vivo*. This conclusion is based on the fact that even if Benzophenone-induced TPO inhibition does occur in the *in vivo* situation, the potential decreases in thyroxine production do not seem to be large enough to elicit any adverse changes in rodent thyroid hormone system.

Regarding estrogenicity, a range of *in vitro* studies and QSAR predictions indicate that Benzophenone itself does not bind to or activate the estrogen receptor, whereas two of its hydroxylated transformation products, 3-OH BP and 4-OH BP do show estrogenic activity *in vitro*. The concern about estrogenic activity of the hydroxylated metabolites, raised by the *in vitro* studies, is supported by results showing estrogenic activity in rodent uterotrophic assays using oral or intraperitoneal dosing of Benzophenone (implying occurrence of first pass metabolism i.e. significant hydroxylation of Benzophenone) as well as in uterotrophic assays with the metabolites 3-OH-BP and 4-OH-BP.

The evaluating MS originally considered that further information was required to clarify the concern on endocrine disrupting effects. Therefore, it prepared a draft decision to request further information on endocrine disrupting effects as follows: Thyroid disrupting activity in vivo (Repeated Dose 28-Day Toxicity Study, in rats by oral route (EU method B.7/OECD 407) and Amphibian Metamorphosis Assay (AMA)(OECD 231)) and Estrogenic activity in fish *in vivo* (Fish Sexual Development Test (FSDT) (OECD 234). The evaluating MS submitted the draft decision to ECHA on 17 March 2014. On 29 April 2014 ECHA sent the draft decision to the Registrant(s) and by June 2014 ECHA received comments from the Registrant(s). The evaluating MS considered the comments received from the Registrant(s) and the dossier update, and updated the draft decision accordingly.

On 23 July 2015 the evaluating MS notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them to submit proposals to amend the draft decision within 30 days of the receipt of the notification. Subsequently, some Competent Authorities of the Member States and ECHA submitted proposals for amendment to the draft decision.

On 28 August 2015 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them to provide comments on those proposals for amendment. The evaluating MS reviewed the proposals for amendment, and based on the Registrant(s)´ comments a Repeated Dose 28-Day Toxicity Study in rats with special investigations of thyroid effects was removed from the draft decision. Furthermore a new test: Larval Amphibian Growth and Development Assay (LAGDA) (OECD 241, July 2015) replaced the request for an AMA (OECD 231), as this test would give more information.

On 7 September 2015 ECHA referred the draft decision to the Member State Committee.

On 28 September 2015, the Registrant(s) provided comments on the proposals for amendment. The Member State Committee took the comments on the proposals for amendment of the Registrant(s) into account.

On 27-29 October 2015 at the Member State Committee meeting a discussion resulted in a change of the information requested in the draft decision for Benzophenone. The initially foreseen experimental study regarding thyroid disrupting activity *in vivo* (LAGDA (OECD 241) / AMA (OECD 231)) and testing for estrogenic activity in fish *in vivo* (Fish Sexual Development Test OECD 234 / Fish Short Term Reproduction Assay (OECD 229) / 21-day Fish Assay (OECD 230) were replaced by a request for further information on fate:

"Available information on metabolism in aquatic non-mammalian vertebrate animals and further information on fate including transformation of Benzophenone with special emphasis on transformation products and kinetics in the aquatic environment and in aquatic toxicity test media."

A unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 29 October 2015. The final decision was issued by ECHA on 30 November 2015.

On 6 June 2016 the Registrant(s) provided further information on the fate of Benzophenone in the environment as requested, based on his updated literature search on the fate of Benzophenone, performed in 2015 and 2016, and two new references were identified. Both newly added references supported the available information data base on fate and behaviour of Benzophenone in the environment which indicates that the compound, once introduced into the environment, will be rapidly removed by different processes.

On March 2017 the evaluating MS assessed Benzophenone and its degradtion products to be readily biodegradable based on the available literature. Furthermore, the substance, its known and expected environmental transformation products and its known metabolites (rodents) are not expected to bioaccumulate in living organisms to any significant extent.

Based on these considerations it is currently evaluated to be very uncertain as to whether this would lead to a regulatory relevant outcome in respect to identification of Benzophenone as a SVHC in accordance with REACH art. 57 (f).

In conclusion, even though there is some residual concern for estrogenic effects and thyroid disruption potential of Benzophenone and its metabolites/degradation products, the evaluating MS finds that currrently there is no straightforward way to follow up on these concerns, and therefore for the time being does not consider this substance to be of priority for further action on the endpoint of endocrine disruption under substance evaluation.

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Benzophenone
EC number:	204-337-6
CAS number:	119-61-9
Index number in Annex VI of the CLP Regulation:	Not applicable

Molecular formula:	$C_{13}H_{10}O$
Molecular weight range:	182
Synonyms:	Diphenyl ketone Diphenylmethanone

Type of substance   ☒ Mono-constituent   ☐ Multi-constituent   ☐ UVCB

Structural formula:
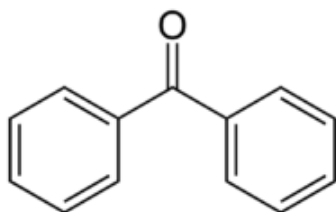
7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Organic solid. White crystals or flakes with a geranium or sweet, rose-like odour
Melting point	48.5 °C (HSDB)
Boiling point	299.49 °C (calculated) (Registrant(s) dossier publicly available at ECHA´s homepage) 305.4 °C (HSDB)
Vapour pressure	0.00257 hPa at 25 °C
Water solubility	137 mg/L (at 25 °C) (HSDB)
Partition coefficient n-octanol/water (Log Kow)	3.18 at 25°C (measured) (HSDB)
Flammability	Not susceptible to ignition on contact with air; however, the flammability range of gaseous Benzophenone is reported to be 0.7 - 5.4 vol%
Explosive properties	Dust can form an explosive mixture with air
Oxidising properties	No oxidising properties
Granulometry	Median diameter D (v, 0.1)=23.18 µm, D (v, 0.5)=228.04µm, D (v, 0.9)=695.93 µm, D (4, 3)=302.12 µm, and D (3, 2)=60.16 µm

	The particle size distribution of Benzophenone crystals was analysed by laser diffraction technology.
Stability in organic solvents and identity of relevant degradation products	Stable in organic solvents based on structural aspects
Dissociation constant	No content of functional groups that are susceptible to dissociation

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☒ **1000- 10,000 t**	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 7

USES	
Uses as intermediate	Intermediate for chemical synthesis
Formulation	Formulation into mixture. Formulation of fragrances, paints, inks, photoinitiators etc.
Uses at industrial sites	Chemical synthesis, photoinitiator in coatings, manufacture of polyester resins, manufacture of fragrances etc.
Uses by professional workers	Professional use of fragrances, polishes and wax blends, photoinitiators, cleaning agents, paints and inks etc.
Consumer Uses	Consumer end-use of, inks and toners and fragrances or fragranced products e.g. cleaning agents or air care products etc.
Article service life	Widespread use of articles with high or intended release (indoor)

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonised classification.

7.6.2. Self-classification

- In the registration(s)*:*
STOT RE 2; H373 May cause damage to organs through prolonged or repeated exposure. (Affected organs: liver, kidney; route of exposure: oral)

Aquatic Chronic 2
H411: Toxic to aquatic life with long lasting effects.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

Acute Tox. 4 ; H302 : Harmful if swallowed.
Skin Irrit. 2; H315: Causes skin irritation
Eye irrit. 2 ; H319 : Causes serious eye irritation
STOT SE 3 ; H335 : May cause respiratory irritation
Carc 2; H351: Suspected of causing cancer.

Aquatic Acute 1 ; H400 : Very toxic to aquatic life
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects
Aquatic Chronic 3; H412: Harmful to aquatic life with long lasting effects

And "Not classified".

7.7. Environmental fate properties

Data on Benzophenone in the environment indicate that the substance, once introduced into the environment, will be rapidly degraded via both biotic and abiotic processes. The Registrant(s) concluded that Benzophenone is readily biodegradable, and the evaluating MS supports this conclusion. Furthermore the Registrant(s) concluded that the substance is not bioaccumulative based on the available information, and the evaluating MS also supports this conclusion. Abiotic degradation (i.e. photolysis) of Benzophenone yields hydroxylated aromatic structures.

Very little experimental data on biotic degradation pathways are available. Based on QSAR model predictions regarding ready biodegradability and degradation time frames and other prediction tools concering biodegradation pathways and formation of biodegradtion metabolites as well as the few experimental studies on the biotic degradation it is highly likely that Benzophenone is biodegraded into more hydrophilic hydroxylated Benzophenone metabolites and phenol and hereafter ultimately degraded. A literature study has in addition provided evidence for likely photolytic formation of 3- and 4-hydroxybenzophenone in surface water. Based on the non-test data assessed by the evaluating MS and the scarce available experimental data it seems that degradation products of Benzophenone include hydroxylated aromatic structures, including the estrogenic 3- and 4- hydroxyl-Benzophenone which, however, all are non-bioaccumulative and also likely quickly to be further ultimately biodegraded.

7.7.1. Degradation

Available information from open literature indicates that Benzophenone may be transformed by photolysis and microbes in aquatic systems (Hayashi *et al.*, 2006; Chen *et al.*, 2015; Fujii & Kikuchi, 2005). No information on degradation on Benzophenone in soil is available. Direct or indirect exposure of the soil compartment to Benzophenone or its degradation metabolites are not to be expected to be significant as they are not used in a way so that soil will be directly

exposed and because they don't have a high potential for adsorption to STP sludge which may be deposited on soil.

7.7.1.1. Degradation rates

Benzophenone has been shown to be photodegraded at low concentrations (µg/L) in pure water as well as in non-polluted surface water (Hayashi *et al.*, 2006 and Chen *et al.*, 2015). Half-life times $T_{1/2}$ for the photolytic degradation of Benzophenone of ~16 hours in pure water, and ~9-11 hours in non-polluted surface water were determined in laboratory. The removal of Benzophenone was faster in the presence of Cl^-, Fe^{3+}, and humic acids. The photodegradation rate of Benzophenone decreased as the initial Benzophenone concentration in water was increased following an exponential function. In laboratory tests simulating the natural conditions, the presence of Benzophenone analogues reduced the photodegradation rate of Benzophenone while the presence of Benzophenone non-analogue did not influence the degradation rate significantly (Chen *et al.*, 2015).

Microbial degradation of Benzophenone in the aquatic environment has been investigated in two studies. The result of a ready biodegradability (OECD TG 301 F) test from 1997 indicates that Benzophenone was readily biodegraded by microbes in natural non-polluted surface water samples, as it showed 66-84 % oxygen consumption after 28 days. In another laboratory study significant Benzophenone-degrading activity was observed using an enriched culture obtained from activated sludge. It was found that the degrading activity was increased through subculturing indicating the enrichment of Benzophenone-degrading microbes in the culture. Isolated Benzophenone-degrading microbes degraded more than 95 % of Benzophenone (100-1000 mg/L) as a sole carbon source within several days; ca. 20 % of Benzophenone were degraded within 40 days at a concentration of 5000 mg/L (no degradation observed at 10000 mg/L after 40 days) (Fujii & Kikuchi, 2005).

In conclusion, data on Benzophenone in the environment indicates that the substance, once introduced into the aquatic environment under most environmental conditions, will be rapidly degraded via both biotic and abiotic processes. Based on these data the Registrant(s) concluded the substance is readily biodegradable, and the evaluating MS can support this conclusion.

7.7.1.2. Degradation pathway

Degradation products of Benzophenone in water and sediment have been examined in two studies.

For abiotic degradation Hayashi *et al*. (2006) identified 3-hydroxy-Benzophenone (3-OH-BP) and 4-hydroxy-Benzophenone (4-OH-BP) as photolytic degradation products when an aqueous solution of Benzophenone was irradiated with sunlight. Hydrolysis of this substance is not expected thus no degradation products of this process are identified.

Very little information regarding the biotic degradation pathway has been available to the evaluating MS. One study on microbial degradation of Benzophenone using an enriched culture obtained from activated sludge found phenol after 4 days in the culture. Phenol was not observed at the beginning of the study (Day 0) or at study termination (Day 7). No other metabolites were mentioned in the paper. However, the evaluating MS notes that on Day 0 a small peak at a lower retention time (more hydrophilic) compared with Benzophenone was present. According to the authors the analysis of metabolites of Benzophenone biotic degradation suggested that Benzophenone was degraded into hydrophilic compounds with very low molecular mass via conversion to phenol (Fujii and Kikuchi, 2005). As Benzophenone was rapidly degraded in this study (no Benzophenone was detected at Day 4 or 7) and measurements of degradation metabolites were only performed at Day 0. 4, and 7, it is difficult to conclude more than that Benzophenone is degraded into hydrophilic compounds

including phenol and hereafter ultimately degraded. Degradation products were neither identified nor characterised in the study on natural degradation by Chen *et al.* (2015*).

In order to get a better understanding of the biodegradation products of Benzophenone the evaluating MS used the publicly available OECD QSAR Application Toolbox, i.e. the OECD Toolbox v. 3.4 Microbial Metabolism Simulator. Here 30 chemical structures were predicted to be formed under biotic degradation hereof eight aromatic substances (see Appendix 7.16 for further information on these). The OECD Toolbox Microbial metabolism simulator does not suggest the degradation pathway but identifies only likely degradation metabolites qualitatively. These included hydroxylated metabolites of Benzophenone (e.g. (2,3-dihydroxy-phenyl)-phenyl-methanone; (3,4-dihydroxy-phenyl)-phenyl-methanone; (2,3-dihydroxyphenyl)(3,4-dihydroxyphenyl)-methanone; and Bis(2,3-dihydroxy-phenyl)-methanone).

In order to assess the degradation pathway the evaluating MS also used the publicly available Pathway Prediction System provided by EAWAG Biocatalysis/Biodegradation Database[2]. According to this Benzophenone is hydroxylated into 2,3-dihydroxy Benzophenone (as also predicted by the OECD Toolbox) and further to succinic acid via several pathways.

The evaluating MS also consulted the publicly available KEGG Pathway Predictor[3] for further information on the biotic degradation pathway of Benzophenone. However, the KEGG Pathway Predictor could not be used to assess the degradation of Benzophenone as Benzophenone was not included in the database. The closest related substance was an ester (i.e. phenyl benzoate) which is not relevant for Benzophenone, as Benzophenone is not an ester.

In conclusion hydroxylated degradation products are likely to be formed, and in most environmental conditions this will be the result of a rapid environmental degradation of Benzophenone. These degradation products are, like the parent compound Benzophenone, also rapidly degradable under most environmental conditions and they have in addition all a lower log Kow value than that of Benzophenone and hence a lower bioaccumulative potential.

7.7.2. Environmental distribution

Not evaluated.

7.7.3. Bioaccumulation

The measured bioconcentration factor of Benzophenone is between 3.4 and 12 at concentrations of 0.3 mg/L and 0.03 mg/L, respectively, when exposed to *Oryzias latipes* (National Institutes of Health, 2010). Based on these BCF values the potential for bioconcentration in aquatic organisms is low.

The logKow is 3.18 (experimental at 25°C) and logKoa is 7.3 (according to DK QSAR DB). Thus the two partitioning coefficients fulfil the screening triggers for potentially bioaccumulative substances in the terrestrial environment, as the estimated logKow values > 2 and the estimated Log Koa values > 6 (Kelly *et al*., 2007). However, based on data on *in vitro* rat hepatocytes (Nakagawa *et al*., 2000) and *in vivo* data on male SD rats (Stocklinski *et al*.,

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[2] http://eawag-bbd.ethz.ch/

[3] http://www.genome.jp/tools/pathpred/

1980) the evaluating MS concludes that Benzophenone is significantly biotransformed in rats. No data are available about the metabolisation rate in fish but the low measured fish BCF value indicates that this is fast in Japanese medaka.

In conclusion the substance is not expected to bioaccumulate in animals to any significant extent.

7.8. Environmental hazard assessment

Information about effects related to endocrine disruption is described and discussed in section 7.10. The lowest acute EC/LC_{50} value in aquatic tests is the 72 hours EC_{50} of 3.5 mg/L based on growth rate for algae. Thus the Registrant(s) concluded that the substance is not acutely toxic to organisms in the aquatic compartment. Benzophenone was not chronically toxic to neither fish nor algae according to the Registrant(s). The Registrant(s) concluded that the substance is chronically toxic to freshwater invertebrates (NOEC of 0.2 mg/L) resulting in a CLP classification of Aquatic Chronic Toxicity category 3 (as the substance is rapidly degradable and not bioaccumulative). Based on the available information, the evaluating MS supports this conclusion.

No studies on toxicity to soil macroorganisms have been submitted by the Registrant(s) as direct or indirect exposure of the soil compartment is not expected to be significant.

7.8.1. Aquatic compartment (including sediment)

The effect of Benzophenone has been investigated in all trophic levels in the aquatic compartments. Due to the rapid transformation of Benzophenone in the aquatic environment including both biotic and abiotic degradation as well as expected metabolism both Benzophenone and its transformation products are discussed in this section when relevant.

7.8.1.1. Fish

Acute toxicity to fish (*Pimephales promelas*) was investigated in a study conducted comparable to OECD guideline 203 under flow-through conditions. In the duplicate tests 96 h LC_{50} a mean 96 h LC_{50} and EC_{50} value of 14.75 mg/L were observed.

Based on this the Registrant(s) concluded that the substance is not acutely toxic to fish (LC_{50} of 14.75 mg/L) and based on the available information, the evaluating MS supports this conclusion.

Sub-chronic toxicity of Benzophenone to early life stage of fathead minnows (*Pimephales promelas*) was investigated in a seven-day study conducted similar to OECD Guideline 212 under flow through conditions. NOEC (7 d) values, based on mortality and sublethal effects (growth), were 5.86 mg/L and 2.1mg/L, respectively.

Shioda and Wakabayashi (2000) investigated the effects of Benzophenone on the reproductivity of medaka (*Oryzias latipes*). After two weeks of exposure of male medaka to Benzophenone the male fish were moved to their original group and kept together with two females. The numbers of eggs spawned were counted every day for a week. Fertilization of eggs was examined and the numbers of hatchings were counted. Benzophenone did not cause any decrease in the numbers of hatchings and eggs even in the highest concentration (10 µmol/L ~ 1.8 mg/L).

No experimental data on the metabolism of Benzophenone in fish has been available to the evaluating MS. However, it is expected that Benzophenone will be metabolised similar in fish as in rats i.e. metabolism will include the metabolite p-hydroxybenzophenone (this metabolite seem to have an estrogenic potential, see further in Section 7.10).

The Registrant(s) concluded the substance is not chronic toxic to fish (NOEC of 2.1 mg/L).

7.8.1.2. Aquatic invertebrates

In a 48 hours acute toxicity study on *Daphnia magna* according to OECD 202 immobilisation of the daphnids was assessed. The results obtained from this study reveal an EC_{50} of 6.78 mg/L and a NOEC of 4.47 mg/L.

The Registrant(s) concluded the substance is not acutely toxic to freshwater invertebrates (LC_{50} of 6.78 mg/L) and based on the available information, the evaluating MS supports this conclusion.

In a 21 days long-term reproduction study performed according to OECD Guideline 211 and using *Daphnia magna* as test organism, the 21-d EC_{50} and NOEC values were determined to be 1.1 and 0.20 mg/L, respectively.

Jubeaux *et al*. (2012) conducted laboratory tests to assess vitellogenin changes in male *Gammarus fossarum* after exposure to chemical stress. The males were exposed among others to Benzophenone for 21 days. At the end of exposure and for each replicate 15 males (5 randomly collected in each replicate) were individually weighted and vitellogenin measured via HPLC/MS/MS. At the end of exposure, no mortality was observed (survival rates >80 %). Benzophenone did not induce vitellogenin production in males at concentrations ranging from 0.001 to 1000 µg/L.

The Registrant(s) concluded that the substance is chronic toxic to freshwater invertebrates (NOEC of 0.2 mg/L) resulting in a CLP classification of Aquatic Toxicity category 3 (as the substance is rapidly degradable) and based on the available information, the evaluating MS supports this conclusion.

7.8.1.3. Algae and aquatic plants

In a 72 hour toxicity study, the cultures of *Pseudokirchenerella subcapitata* were exposed to Benzophenone in accordance with OECD Guideline 201. The 72 hour EC_{50} and NOEC values based on growth rate were determined to be 3.5 and 1.0 mg/L, respectively.

The Registrant(s) concluded that the substance is not toxic to algae (LC_{50} of 3.5) and based on the available information, the evaluating MS supports this conclusion.

7.8.1.4. Sediment organisms

No studies on toxicity to sediment macroorganisms have been submitted by the Registrant(s) as Benzophenone is readily biodegradable. Therefore, it can be assumed that the substance will be extensively biologically degraded within the STP process. Furthermore, for substances not passing the STP-process but being readily biodegradable, it can be assumed that they will be also biologically degraded in the surface water within a short timeframe under most environmental conditions. As furthermore the degradation products of Benzophenone all have a lower log Kow and will be more rapidly degraded than Benzophenone significant exposure of sediment organisms to Benzophenone and its degradation products are unlikely.

7.8.1.5. Other aquatic organisms

Canesi *et al*. (2007) investigated the effects of Benzophenone on several hemocyte parameters (lysosomal membrane stability (LMS), phagocytosis, lysozyme) of the marine bivalve *Mytilus galloprovincialis*. Lysosomal membrane stability, which proved to be the most sensitive effect parameter, was evaluated by the Neutral Red Retention time assay. The NOEC and LOEC (LMS) for Benzophenone were determined to be 0.01 (=1.82 µg/L) and 0.1 µM (=18.2 µg/L),

respectively. The EC_{50} for Benzophenone exposure was determined to be 8.535 µM (=1.55 mg/L). It is noted that these effect concentrations in mussel address a biochemical response parameter which is traditrionally not used for hazard and risk assessment.

7.8.2. Terrestrial compartment

No studies on toxicity to soil macro-organisms have been submitted by the Registrant(s) as direct or indirect exposure of the soil compartment is not likely to be significant.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

When tested *in vitro* in rat hepatocytes, Nakagawa *et al*. (2000) found that Benzophenone was converted to at least three metabolites, 4-OH-BP, its sulfate conjugate and benzhydrol. Furthermore, in male SD rats administered Benzophenone in corn oil by gavage 4-OH-BP was isolated from the urine, and accounted for approximately 1 % of the administered dose (Stocklinski *et al*., 1980).

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

Not evaluated.

7.9.4. Repeated dose toxicity

The evaluating MS has examined the performed repeated dose toxicity studies, in the evaluation of Benzophenone. In the table 8 below an overview of relevant studies is presented, and the studies are decribed in detail below the table.

Table 8 Overview of experimental studies on repeated dose toxicity after oral administration

Study	Method	Results	Remarks
NTP (2000)	rat (F344/N) male/female subchronic (oral: feed) 0, 1250, 2500, 5000, 10000 or 20000 ppm Benzophenone IUPAC name (m: 0, 75, 150, 300, 700 or 850 mg/kg bw/d; f: 0, 80, 160, 300, 700 or 1000 mg/kg bw/d) (nominal in diet) Exposure: 14 weeks (Continuous via diet)	no NOAEL identified: (male/female) based on: test mat. (Foci of tubule regeneration were increased relative to the controls in exposed males and females of all groups. Based on these findings, a NOAEL for kidney changes was not reached in either males or females. Exposure-related increases in liver weights were attributed to hypertrophy and/or cytoplasmic	IUPAC name: diphenyl methanone.

	equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	vacuolization of hepatocytes in all groups.)	
NTP (2000)	mouse (B6C3F1) male/female subchronic (oral: feed) 0, 1250, 2500, 5000, 10000 or 20000 ppm(m: 0, 200, 400, 800, 1600 or 3300 mg/kg bw/d; f: 0, 270, 540, 1000, 1900 or 4200 mg/kg bw/d) (nominal in diet) Exposure: 14 weeks (Continuous via diet) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	no NOAEL identified: (male/female) based on: test mat. (The gross (increased organ weights) and microscopic (hepatocellular hypertrophy) liver changes associated with Benzophenone administration in males and females were accompanied by Benzophenone-induced increases in the activity of pentoxyresorufin dealkylase, an enzyme linked to the cytochrome P450 2B isomer.)	IUPAC name: diphenyl methanone. EC name: Benzophenone
Burdock *et al.* (1991)	rat (Sprague-Dawley) male/female subchronic (oral: feed) 0, 20, 100 and 500 mg/kg bw/d (intended intake) m: 0, 18.6, 91.7 and 485 mg/kg	NOEL: ca. 20 mg/kg bw/day (nominal) (male/female) based on: test mat.	IUPAC name: diphenyl methanone. EC name: Benzophenone

Benzophenone was administered in the diet to rats at target dose levels of 20 mg/kg bw/day for 90 days and 100 or 500 mg/kg bw/day for 28 days (Burdock *et al.*, 1991). Body weights and food consumption were measured weekly; haematology, clinical chemistry and urinalysis values were obtained at 4 weeks and at the end of the study. Gross and microscopic pathological examinations were conducted and organ weights were recorded. Treatment-related changes occurred in erythrocyte count, haemoglobin, haematocrit, bilirubin, total protein and albumin at the mid- and high-dose levels, although all changes did not occur in both groups in both sexes.

There were indications of increased absolute and relative liver and kidney weights in the mid- and high-dose groups, but this was not statistically consistent for absolute kidney weights. Histopathology of the liver in the mid- and high-dose groups showed hepatocellular enlargement with an associated clumping of cytoplasmic basophilic material around the central vein. A no-effect level was demonstrated at 20 mg/kg bw for 90 days of administration (Burdock *et al.*, 1991). The evaluating MS concurs with this evaluation.

From two NTP 14- week oral studies (NTP 2000) with dosing of F344/N rats and B6C3F1 mice with 0, 1250, 2500, 5000, 10000 or 20000 ppm continuously via diet no NOAEL values could be identified due to effects even at the lowest dose. These effects in the low dose in male rats included: a decreased activity of alkaline phosphatase (ALP) (day 22, week 14, p<0.01), increased absolute and relative kidney weights (p<0.01), increased incidence of protein casts in renal tubules (p<0.05), increased severity of renal tubule regeneration (NS), and increased incidence of cytoplasmic vacuolisation of hepatocytes (p<0.01).These effects in female rats included: lower body weight (p<0.01) and body weight gain (p<0.01), decreased activity of ALP (p<001), increased bile salt concentrations 8p<0.05), increased absolute liver weight (p<0.01), increased relative weights of liver and kidney (p<0.01), increased incidence of renal tubule regeneration (p<0.05). In the mouse study the low dose effects included increased absolute and relative liver weights in both sexes (p<0.01) and increased incidence of centrilobular hypertrophy in the liver (p<0.05 for males and p<0.01 for females). The LOAEL for rats and mice was given with 75-80 mg/kg bw and 200-270 mg/kg bw, respectively. (NTP, 2000). The evaluating MS concurs with this evaluation.

For the dermal and inhalation route there are no data available.

Results from these studies, which are relevant for the evaluation of possible endocrine disrupting effects of Benzophenone, are presented and discussed in section 7.10.

The following information is taken into account for any hazard / risk assessment:

Subchronic oral (diet) study in rats, 90 days: NO(A)EL 20 mg/kg bw (Burdock *et al*., 1991) From two other 14 w oral studies with dosing of F344/N rats and B6C3F1 mice with 0, 1250, 2500, 5000, 10000 or 20000 ppm continuosly via diet no NOAEL values could be identified due to effects even at the lowest dose. The LOAEL for rats and mice was given with 75-80 mg/kg bw and 200-270 mg/kg bw, respectively (NTP, 2000).

For the dermal and inhalation route there are no data available.

NOAEL: 20 mg/kg bw/day (subchronic; rat)

Target organs: digestive: liver

7.9.5. Mutagenicity

The results of genotoxicity assays with Benzophenone showed no evidence of genotoxicity both in *in vitro* and *in vivo* tests.

In one assay, the use of human recombinant P450 enzyme preparations, including P450 family 1 enzymes, in a *Salmonella typhimurium* umu gene expression assay with Benzophenone and two metabolites, benzhydrol and p-benzoylphenol, produced dose-related increases in gene expression (Takemoto *et al*., 2002). However, this assay is not an OECD guideline assay and moreover Benzophenone was negative in the bacterial *in vitro* OECD guideline test, *Salmonella typhimurium* gene mutation assay. Benzophenone was also negative in another bacterial assay, the unscheduled DNA synthesis test, as well as in the OECD guideline test using mammalian cells, the mouse lymphoma assay. Two reported *in vivo* micronucleus tests of Benzophenone in mice gave negative results.

Based ion this the evaluating MS has taken the overall conclusion that Benzophenone is not genotoxic.

Table 9 Overview of experimental *in vitro* genotoxicity studies.

Method	Results	Remarks	Reference
Bacterial reverse mutation assay (Ames test, gene mutation) (metabolic activation.: with and without) Doses: 0, 1, 3, 10, 33, 100, 166, 333 and 1000 ug/plate Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results:negative with and without metabolic activation Test results: negative in strains TA 1535, TA 1537, TA 98 and TA 100. No cytotoxicity but tested up to precipitating concentrations.	2 (reliable with restrictions) Key study Experimental result **Test material: Benzophenone**	NTP (2006)
Mammalian cell gene mutation assay (mouse lymphoma L5178Y cells) (metabolic activation.: with and without) Doses: 35 - 170 µg/mL Equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test)	Evaluation of results:negative with and without metabolic activation Test results: negative for mouse lymphoma L5178Y cells (all strains/cell types tested)	2 (reliable with restrictions) key study experimental result **Test material: Benzophenone**	Seifried *et al.* (2006a)
Bacterial reverse mutation assay (WP2 Mutoxitest: gene mutation) Strains: *E. coli* WP2 uvr A pKM 101(with and without metabolic activation) Other strains: *E. coli*, other: IC203 (with and without metabolic activation) Doses: five to six concentrations were tested, highest non-toxic dose was 200 µg/plate The WP2 Mutoxitest is used for the detection of oxidative mutagenicity. No S. *typhimurium* strains tested in this assay.	Evaluation of results: negative Test results: negative for *E. coli*, other:WP2 uvrA/pKM101 and IC203 (all strains/cell types tested) with and without metabolic activation.	2 (reliable with restrictions) key study experimental result **Test material: Benzophenone**	Martinez *et al.* (2000)

Bacterial reverse mutation assay (Ames test, gene mutation) S. *typhimurium* strains: TA98, TA100, TA1535, TA1537 and TA1538 (with and without metabolic activation) Doses: a total of 5 concentrations was selected (10 - 2000 µg/plate) Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative results for all strains tested both with and without metabolic activation	2 (reliable with restrictions) supporting study experimental result **Test material: Benzophenone**	Seifried *et al.* (2006b)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* (DNA damage and/or repair). *E. coli*, other: W3110 (Pol A+) and p3478 (Pol A-) (With and without metabolic activation). Doses: 500 µg The test is based on the finding that cells exposed to agents that modify their DNA tend to protect themselves by excising the altered DNA portion and then resynthesizing the correct sequence.	Evaluation of results: negative both with and without metabolic activation Test results: negative for *E. coli*, other: W3110 (Pol A+) and p3478 (Pol A-)(all strains/cell types tested)	2 (reliable with restrictions) key study Experimental result **Test material: Benzophenone**	Fluck *et al*. (1976)
SOS/umu test (DNA damage and/or repair)-Bacterial test S. *typhimurium* TA 1535/ pSK1002 (with metabolic activation) Doses: 1 - 1000µM The genotoxicity of Benzophenone was determined by measuring the induction of umu gene expression in *S. typhimurium* TA1535/pSK1002 or O-acetyltransferase-overexpressing strain NM2009.	Evaluation of results: Positive Test results: positive for *S. typhimurium* TA 1535 pSK1002 (all strains/cell types tested) ; met. act.: not applicable	2 (reliable with restrictions) Supporting study Experimental result **Test material: Benzophenone**	Takemoto *et al*. (2002)

Table 10 Overview of experimental *in vivo* genotoxicity studies.

Method	Results	Remarks	Reference
Micronucleus assay (chromosome aberration), mouse (B6C3F1) male Intraperitoneal administration 200, 300, 400 and 500 mg/kg bw (injection), solvent was corn oil OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results:negative Test results: Genotoxicity: negative (male); Toxicity: no effects	1 (reliable without restriction) key study experimental result **Test material: Benzophenone**	NTP (2000b)
Micronucleus assay (chromosome aberration) mouse (CBA and NMRI) male Intraperitoneal administration Experiment 1: 500, 1000 and 2000 mg/kg bw. Experiment 2: 100, 250, 400 and 600 mg/kg bw. Equivalent or similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: negative Test results: Genotoxicity: negative (male); Toxicity: yes in Experiment 1.	2 (reliable with restrictions) key study experimental result **Test material: Benzophenone**	Abrahamsson-Zetterberg & Svensson (2011)

7.9.6. Carcinogenicity

The data from long-term studies with Benzophenone via dietary exposure in mice and rats conducted by (NTP 2006) were examined by the evaluating MS.

Two studies using dermal exposure were also included in the evaluation (Stenbäck and Shubik, 1974 and Stenbäck, 1977).

The studies and the result of the examination are described in details below. Results from the long-term studies which are relevant for the evaluation of possible endocrine disrupting effects of Benzophenone, are presented and discussed in section 7.10.

7.9.6.1. NTP study in mice

A carcinogenesis bioassay of Benzophenone in mice was conducted by the National Toxicology Program (NTP 2006) following a guideline similar to OECD TG 451 and in accordance with the GLP principles.

Groups of 50 male and 50 female $B6C3F_1$ mice were exposed via the diet containing 0, 312, 625, or 1250 ppm Benzophenone for 105 weeks. These dietary concentrations corresponded to doses of 40, 80, and 160 mg Benzophenone/kg bw/day for males and 35, 70, and 150 mg

Benzophenone/kg bw/day for females. The high dose was set based on the minimal toxicity observed at this level in the 14-week study (NTP 2000) with regard to liver weight.

Survival of treated mice was similar to that of the controls, except for the high-dose females for which a not statistically significantly decreased survival towards the end of the study was recorded.

7.9.6.1.1. Non-neoplastic changes in mice.

Mean body weights of Benzophenone treated male mice were similar to the controls throughout the study. Mean body weights of female mice from the high-dose group were less than those of the controls from week 37, of females from the middle-dose group were less during the entire study period, and of females from the low-dose group were less after week 86.

Feed intake of the treated mice of both sexes was similar to that of the controls throughout the study.

In the *liver* a statistically significantly increased incidences of the hypertrophy of hepatocytes were reported in all treated male and female groups (males: 0/50, 44/50, 50/50, 48/50; females: 0/50, 29/50, 44/50, 37/50, in the control, low-, middle- and high-dose groups, respectively). An increase of multinucleated hepatocytes (0/50, 41/50, 47/50, 48/50 in the control, low, middle and high-dose groups, respectively) and of an active chronic inflammation (33/50, 47/50, 44/50, 42/50 in the control, low, middle and high-dose groups, respectively) were reported for all treated male groups.

In the *kidneys* a statistically significantly increased severity of nephropathy was reported for all treated male groups (mean severity scores: 1.2, 1.4, 1.7, 3.0 in the control, low, middle and high-dose groups) and increased incidences of nephropathy were reported for all treated female groups (21/50, 33/50, 31/50 and 30/50 in the control, low, middle and high-dose groups, respectively).

In the *spleen* hyperplasia of lymphoid follicles was statistically significantly increased in all treated groups of both sexes, except the high dose females (males: 17/50, 31/50, 34/50, 32/50; females: 24/50, 36/50, 37/50, 22/50, in the control, low and middle and high-dose groups). Furthermore, in this organ the incidence of the hematopoietic cell proliferation was statistically significantly greater than that of the controls in all female groups (16/50, 35/50, 32/50, 27/50 in the control, low-, middle- and high-dose groups, respectively).

In the *nose* the incidence of metaplasia of the olfactory epithelium was statistically significantly increased in the high-dose group in both sexes.

In *testes* the incidence of mineralization was increased (0/50; 1/50; 4/50; and 12/50 in the control, low-, middle- and high-dose groups, respectively), and was statistically significantly increased in the high-dose group.

7.9.6.1.2. Neoplastic changes in mice.

Neoplastic changes were recorded in the liver of treated male and female mice.

In males, there was a positive trend for all liver tumours. The incidences of **hepatocellular adenoma** were statistically significantly increased in the 2 highest dose groups. The incidences of this benign tumour (11/50, 15/50, 23/50, 23/50 in the control, low-, middle- and high-dose groups, respectively) in all male treated groups were at the upper boundary or exceeded the range for historical incidence from the same laboratory (12-30 %) and the ones reported in the literature for the same strain (Chandra & Frith, 1992).

In females the incidences of **hepatocellular adenoma** in the middle- and high-dose group were not statistically significantly increased when compared to the control group (5/50, 4/50, 10/50, 8/50 in the control, low-, middle- and high-dose groups, respectively) but exceeded the upper value of the range of historical controls from the same laboratory (6 %-12 %) and were also greater than the incidences reported in the literature for the same strain (Chandra & Frith 1992, Maronpot 1999).

The combined incidences of malignant liver tumours in males (**hepatocellular carcinoma** and **hepatoblastoma**) were jointly (8/50, 6/50, 7/50, 9/50 in the control, low-, middle- and high-dose groups, respectively) in the treated male groups were not significantly different from those in the control group. Statistically significant differences was found for **combined** incidences of hepatocellullar adenoma, carcinoma and hepatoblastoma in the high-dose males (29/50, p=0.027, Poly-3 test reported by the authors of the study; p= 0.0446, Fisher's exact test performed by the Danish Environmental Protection Agency during the substance evaluation).

In females, sporadic and not statistically significantly different from the controls incidences of **hepatocelluar carcinoma** were recorded in the treated female groups (0/50, 1/50/0/50, 1/50 in the control, low-, middle- and high-dose groups, respectively). No hepatoblastomas were reported. The **combined** incidences of hepatocellular adenomas and carcinomas were not statistically significantly increased compared to the controls.

The malignant tumour of the haematopoietic system **histiocytic sarcoma** was reported in the female middle and high dose groups. The incidences of this tumour (0/50, 0/50, 5/50, 3/50 in the control, low-, middle- and high-dose groups, respectively) showed a positive trend and there was a statistically significant increase in the middle-dose group as compared to the control group.

7.9.6.1.3. Discussion of the results from the NTP study in mice

The study by NTP in B6C3F$_1$ mice is considered a reliable study, as it is conducted in compliance with a guideline similar to OECD TG 451 and GLP principles.

Concerning non-neoplastic changes, the increased incidences of metaplasia of the olfactory epithelium in male and female mice from the high-dose group could be a result of a repair of the earlier damage, as suggested by the authors of the study. Increased incidences of non-neoplastic changes in the liver (hyperthrophy of hepatocytes in all treated male and female groups, multinucleated hepatocytes and an active chronic inflammation in all treated male groups) have been recorded. The evaluating MS considered hyperthrophy of hepatocytes to be related to the treatment with Benzophenone. Hyperthrophy of hepatocytes is an adaptive change associated with toxicity (Turton and Hooson, 1998), and by an increased number of multinucleated hepatocytes it is indicative of a regenerative activity of the liver, which may be due to increased cell division or suppression of cell division (Kostka *et al*., 2000).

The authors of the study concluded that there was some evidence of carcinogenic activity of Benzophenone in male B6C3F$_1$ mice based on increased incidence of hepatocellular neoplasms, and that the increased incidences of hepatocellular adenoma in female mice may have been related to Benzophenone exposure. Also, the authors of the NTP study concluded that there was some evidence of carcinogenic activity of Benzophenone in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcoma.

The incidences of hepatocellular adenoma in the male mice showed a positive trend. No NOAEL could be identified for incidences of adenoma. With regard to this change the low dose is a LOAEL (40 mg Benzophenone/kg bw/day).

In females incidences of hepatocellular adenomas were increased in the middle – and the high-dose groups. The incidences were not statistically significantly increased compared to the concurrent control but exceeds the historical controls from the same laboratory. It is noted

that the incidence of this tumour in the concurrent control was also in the upper value of historical controls (12 %). Therefore, the evaluating MS considers that the hepatocellular adenoma in female mice should be regarded a significant effect indeed related to Benzophenone treatment.

The B6C3F$_1$ strain of laboratory mice has been used in many carcinogenicity studies particularly by NTP and a considerable amount of data on spontaneous tumour incidences in this strain exist. This mouse strain carries hepatocellular tumor susceptibility loci that result in a high susceptibility to chemically induced cellular hepatocarcinogenesis (Gariboldi *et al.*, 1993; Manenti *et al.*, 1994).

Other historical data for hepatocellular adenoma, show that this tumour is a common hepatic neoplasm in B6C3F$_1$ mice with spontaneous incidence of about 30% in males and 15% in females (Harada *et al.*, 1996 as cited in Maronpot 1999).

In relation to hepatocellular adenoma the evaluating MS noted that there is a general consensus in the scientific community backed by a considerable body of evidence that hepatic tumours in mice when induced by non-genotoxic compounds can be considered as irrelevant for human risk assessment (Gold & Slone, 1993; Carmichael *et al.*, 1997; Boobis *et al.*, 2006; Holsaple *et al.*, 2006; Billington *et al.*, 2010).

Malignant hepatic tumours recorded in mice include hepatoblastoma and hepatocarcinoma in male mice, and hepatocarcinomas in female mice. With regard to the incidence of hepatoblastoma, a positive trend in the incidence of this tumour reported by the authors of the study implies its relation to the treatment. The incidences were not statistically significantly different from that in the controls but they exceed historical incidences. Hepatoblastoma is an uncommon tumour even in older B6C3F$_1$ mice, although its incidence rate in the control B6C3F$_1$ mice has increased in NTP studies (i.e. 0.3 % between 1986 and 1992,and 1.6 % between 1993 and 1999) (Turusov *et al.*, 2002).

The hepatocarcinomas in the treated males occurred at non-significant levels, within historical controls data in the male B6C3F$_1$ mice (a range of 6-29 %, Maronpot, 1999) and no dose-response was demonstrated. Thus, the evaluating MS considers this tumour to have a low relation to treatment with Benzophenone.

In females, the single incidence of hepatic carcinoma in the low and high dose group could be an incidental, not treatment related finding considering that the incidence of spontaneous hepatocarcinoma in female B6C3F$_1$ mice is highly variable as indicated by the range 0-20 % (Maronpot, 1999).

The evaluating MS notes that hepatocellular adenomas, hepatoblastomas and hepatocellular carcinomas represent a biological and morphological continuum in progression of proliferative lesions.

In relation to carcinogenicity in the haematopoietic system in female B6C3F$_1$ mice the occurrence of histiocytic sarcomas, a rare tumour form, is noted. The incidence reported in the study is statistically significant in the mid-dose, but not at the high dose, (0/50, 0/50, 5/50, 3/50 in the control, low-, middle- and high-dose groups, respectively). The incidences in the mid- and high dose groups exceed historical incidence reported by the authors of the study (0-2 % in 2 year studies with feed controls) and in the literature (0-4 % from Maronpot, 1999). Also, a positive trend in the incidences of this tumour was reported by the authors of the study, and indicate a relation to treatment even if a dose response is not apparent. The concern for a carcinogenic effect to the hematopoietic system is supported by the statistically significantly increased incidence of the hematopoietic cell proliferation in the spleen in all female groups (16/50, 35/50, 32/50, 27/50).

Overall, the evaluating MSCA concurs with the overall conclusion of the authors that Benzophenone shows some evidence of carcinogenic activity in mice under the conditions of the NTP study.

7.9.6.2. NTP study in rats

A carcinogenesis bioassay of Benzophenone in rats was conducted by the National Toxicology Program (NTP, 2006). Groups of 50 male and 50 female F344/N rats were exposed via the diet containing 0, 312, 625, or 1250 ppm Benzophenone for 105 weeks. These dietary concentrations corresponded to doses of 15, 30, and 60 mg Benzophenone/kg bw/day for males and 15, 30, and 65 mg Benzophenone/kg bw/day for females. The high dose was set based on the minimal toxicity observed at this level in the 14-week study (NTP, 2000) with regard to reduction in body weight gain and increases in liver weights in females and males.

Survival of males at the high-dose group was statistically significantly less than that of the control group. Survival of low and middle-dose males and of all treated female groups was similar to that of the control group.

7.9.6.2.1. Non-neoplastic changes in rats

Mean body weights of the high-dose group males were markedly less than those of the controls during the second year of the study, and body weights of all exposed rats were consistently less than the controls throughout the study.

Feed intake in the high-dose male group was less than the controls after week 70. Females from the high-dose group had generally lower feed intakes than the controls throughout the study.

Statistically significant and/or biologically noteworthy non-neoplastic changes were found in the kidney, liver, and thyroid gland.

In the *kidneys* three evaluations of histopathological changes were performed: a standard (of single kidney sections), an extended (of step sections because the standard examination revealed increased incidences of renal tubule adenoma in both sexes and of renal tubule hyperplasia in males), and a combined (of single and step sections).

A statistically significant increase in severity of nephropathy was recorded in a standard evaluation in all treated male groups (1.3, 2.4, 3.3, 3.8 in the control, low-, middle- and high-dose groups, respectively) and in the middle – and high-dose group females (1.1, 1.4, 1.7, 2.0 in the control, low-, middle- and high-dose groups, respectively) but the incidences of this non-neoplastic change in all treated groups of both sexes were similar to the controls. A standard evaluation revealed statistically significantly increased incidences of hyperplasia of transitional epithelium of renal pelvis in all male treated groups (1/50, 11/50, 29/50, 34/50 in the control, low-, middle- and high-dose groups, respectively). Following an extended examination incidences of renal tubule hyperplasia were statistically significantly increased in all male and female treated groups (males: 3/50, 11/50, 30/50/40/50; females: 1/50, 8/50, 10/50, 7/50 in the control, low-, middle- and high-dose groups, respectively) and the severity of this pre-neoplastic lesion was statistically significantly enhanced in the high-dose male group (2.1 *versus* 1.0 in the control group).

In the *liver* the noteworthy non-neoplastic lesions included: centrilobular hypertrophy of hepatocytes with statistically significantly increased incidences in all treated groups of both sexes (males: 0/50, 17/50, 31/50, 19/50; females 0/50, 27/50, 30/50, 33/50 in the control, low-, middle- and high-dose groups, respectively), cystic degeneration with statistically significantly increased incidences in the middle- and high-dose male groups (8/50, 11/50, 20/50, 15/50 in the control, low-, middle- and high-dose groups), chronic active inflammation with statistically increased incidences in the middle- and high-dose male groups and statistically significantly decreased incidences in all treated female groups (males: 22/50,

21/50, 35/50, 33/50; females: 46/50, 38/50, 29/50, 30/50 in the control, low-, middle- and high-dose groups, respectively), and bile duct hyperplasia with statistically increased incidences in all treated female groups (10/50, 35/50, 39/50,40/50 in the control, low-, middle- and high-dose groups).

In the **thyroid gland** statistically significantly lower incidences of C-cell hyperplasia were recorded in all treated groups of both sexes (males: 17/50, 8/50, 8/50, 5/50; females: 34/50, 11/50, 13/50; 8/59 in the control, low-, middle- and high-dose groups, respectively).

7.9.6.2.2. Neoplastic changes in rats

In the rats the kidney and the haemopoetic system were the targets for neoplastic lesions which included renal tumours, mononuclear cell leukemia (MNCL) and histiocytic sarcomas.

In males a combined evaluation of single sections and step sections of the **kidney** revealed a positive trend in the incidences of renal tubule adenoma and a statistically significantly increased incidence of this tumour in the high-dose group (2/50, 2/50, 7/50, 8/50 in in the control, low-, middle- and high-dose groups, respectively). The incidences in the middle and high-dose male groups exceeded the historical control range from the same laboratory (0 – 2 %). In females the renal tubule adenomas were reported solely in the middle-dose group (3/50) and the high-dose group (1/50). Their incidences were not statistically significantly different than in the control group (3/50).

MNCL was recorded in the control and treated groups of both sexes (males: 27/50, 41/50, 39/50, 24/50; females: 19/50, 25/50, 30/50, 29/50 in the control, low-, middle- and high-dose groups, respectively). Statistically significantly increased incidences of MNCL were recorded in the low- and middle-dose male groups, and in the middle-dose female group. The incidences in all treated female groups and in the low- and middle-dose groups of males exceeded the historical incidence from the same laboratory from 2-year feed studies with controls given the same control diet as in this study (males: 30-68 %; females: 12-38 %) and by all routes (males: 22-68 %; females: 12-52 %). The extent of involvement by MNCL expressed as an average staging grade was statistically significantly decreased in all treated male groups (scores: 2.8, 2.4, 2.2, 2.0 in the control, low-, middle- and high-dose groups, respectively).

Histiocytic sarcomas were found in the middle- and the high-dose group of females (0/50, 0/50 1/50, 2/50 in the control, low-, middle- and high-dose groups, respectively). The incidences of this rare neoplasm exceeded the historical incidence from the same laboratory for feed studies (0/460) and for all routes (range 0-2 %). In mammary gland, the incidences of fibroadenoma were statistically significantly decreased in all treated female groups as compared to the control and historical controls. In skin, incidences of keratoacanthoma were decreased in all exposed male groups, the difference reaching a statistical significance in the low- and middle-dose groups. The authors of the study concluded that there was some evidence of carcinogenic activity of Benzophenone in rats based on increased incidence of histiocytic sarcomas.

7.9.6.2.3. Discussion of the long-term study in rats

The study by NTP in F344/n rats (NTP, 2006) is a long–term carcinogenicity study performed according to a guideline similar to OECD TG 451 and in accordance with the GLP principles.

The evaluating MS noted that the F344 strain of laboratory rat is well characterized since it has been the selected rat strain for the National Toxicology Programme (NTP) for over 20 years. Thus considerable amount of data on spontaneous tumour incidences in this strain exist, which is of importance for hazard characterisation.

Concerning non-neoplastic changes and in relation to the statistically significantly increased incidences of centrilobular hepatocellular hypertophy in all treated males and females the

evaluating MS noted that this finding was treatment related. Hepatocellular hypertrophy is an adaptive response (Haschek *et al*., 2010) and as such not an adverse change. The evaluating MS further noted that the recorded centrilobular hepatocellular hypertrophy was consistent with the induction of P450 enzymes reported in the 14-week study (NTP, 2000) and presence of this lesion in the rats in a two-generation reproductive toxicity study (Hoshino *et al.,* 2005). Hypertrophy of hepatocytes was also recorded in all treated groups of male and female mice in the 2-year carcinogenicity study with bezophenone (NTP, 2006). For this non-adverse change a no-effect-level (NOEL) could not be identified in both sexes, and the LOEL was the low-dose corresponding to 15 mg Benzophenone /kg bw/day.

Furthermore, concerning non-neoplastic changes the increased incidences of chronic active inflammation and of cystic degeneration of hepatocytes in the middle and high-dose groups of males and of bile duct hyperplasia in all treated female groups could be a part of response to injury due to exposure to Benzophenone according to the evaluating MS. The lowest BMDL with regard to bile duct hyperplasia in female rats as calculated by the evaluating MS was 1.14 mg Benzophenone/kg bw/day. The finding of statistically significantly decreased incidences of C-cell hyperplasia in the thyroid gland in exposed groups of males and females were considered a treatment related effect since increased thyroid gland C—cell hyperplasia is an age-associated change in rats (Boorman *et al*., 1996). The evaluating MS concurred with this view.

No NOAEL could be identified for incidence of renal tubule hyperplasia in male and female rats in this study and the low dose corresponding to 15 mg Benzophenone/kg bw/day was considered a LOAEL. As the effect was most pronounced in males the evaluating MS applied Benchmark dose approach to the data from males. The lowest Benchmark dose low (BMDL) calculated by the evaluating MS for renal tubule hyperplasia incidences in male rats was 3.6 mg Benzophenone/kg bw/day.

In the case of Benzophenone, kidney lesions indicative of a chronic progressive nephropathy were increased in exposed males and females in the 14-week study and the severity of chronic progressive nephropathy was statistically significantly increased with increasing doses in the treated males in the 2-year study (NTP, 2000). Chronic progressive nephropathy is a common spontaneous disease in rat kidney and the Fisher rats are inclined to develop the disease (Travlos *et al*., 2011). In relation to chronic progressive nephropathy the evaluating MS noted that no NOAEL could be identified for its severity in male rats and the LOAEL was the low-dose corresponding to 15 mg Benzophenone /kg bw/day. In female rats the NOAEL for the severity of chronic nephropathy was the low dose.

The evaluating MS noted that statistically significantly increased incidences of pelvic transitional epithelium hyperplasia in all treated male groups (and slightly but not statistically significantly increased incidence of this lesion in the high dose females) may reflect the enhanced nephropathy. The lowest Benchmark dose low (BMDL) calculated by the evaluating MS for incidences of pelvic transitional epithelium hyperplasia in male rats was 2.9 mg Benzophenone/kg bw/day.

Concerning neoplastic changes and according to the authors:

   a) increased incidences of renal tubule adenoma provide *some evidence of carcinogenic activity* of Benzophenone in male F344/N rats,
   b) MNCL in the male F344/N rats may have been related to Benzophenone exposure, and
   c) there is *equivocal evidence of carcinogenic activity* of Benzophenone in female F344/N rats based on MNCL and histiocytic sarcoma.

The authors report a positive trend in treated males in the incidences of renal tubule adenoma with a statistically significanctly increased incidence in the high-dose group, and a statistically significantly increased incidence of renal tubule hyperplasia in all treated groups of males and females. Furthermore, they report statistically significantly increased incidences of MNCL in the low- and middle-dose groups of males, and in the middle-dose group of females. Additionally,

they report single incidences of histiocytic sarcoma in the middle- and high dose groups of females.

In relation to renal tubule adenoma in male F 344 rats the evaluating MS noted that spontaneous renal neoplasms in the rat are uncommon (Chandra *et al*., 1993). Incidences of renal cell adenomas as low as 0.38 % and 0.19 % were reported for male and female F 344 rats, respectively (Chandra *et al*., 1993).

In the NTP study in rats a middle dose corresponding to 30 mg Benzophenone/kg bw/day can be identified as a NOAEL for renal tubule adenoma in male rats, according to the evaluating MS. The lowest Benchmark dose low (BMDL) calculated by the evaluating MS for renal tubule adenoma incidences in male rats was 16.6 mg Benzophenone/kg bw /day.

The evaluating MS noted that in male rats the relation to the treatment of renal tubule adenoma is suggested by the fact that the first incidence was recorded in the high-dose group, that the incidences in the treated male groups exceeded the historical incidence for 2-year feed studies with control given NTP-2000 diet (range 0-2 % as reported in NTP, 2006), and that a positive trend in the incidences of renal tubule adenoma in males has been reported by the authors of the study. The evaluating MS also noted that further support for relation to Benzophenone treatment of this tumour inmale rats is provided by statistically significantly increased incidences of renal tubule hyperplasia in all treated male groups. This is because renal tubule hyperplasia, adenoma, and carcinoma represent a continuum in the progression of proliferative lesions of the renal tubule epithelium.

According to the evaluating MS the relation to treatment of the renal tubule adenoma in male rats is further supported by an association between treatment related exacerbation of severity of chronic progressive nephropaty in the 90-day studies and increased incidence of renal tubule tumours at 2 years (Travlos *et al*., 2011).

Benzophenone has been considered to exacerbate chronic progressive nephropathy based on the morphological changes in the kidney in the 14-week and the 2-year study (NTP, 2000; 2006). Thus involvement of chronic nephropaty in development of the renal tubule tumours in the NTP study is plausible according to the evaluating MS. It has to be noted that it has been suggested that renal tubular tumours arising in association with chemically induced chronic progressive nephropathy should have no relevance for species extrapolation in human risk assessment (Travlos *et al*., 2011) as chronic progressive nephropathy has no counterpart in humans (Hard *et al*., 2009).

In relation to MNCL in F-344 rats of both sexes the evaluating MS noted that this hemolymphoreticular neoplasm is unique to the rat and is only common in this strain. MNCL has not been found in other mammalian species (e.g. mice and hamsters) and no histologically comparable tumour is found in humans (Caldwell, 1999). MNCL occurs in untreated, aged F-344 rats at a high and variable rate, it is uncommon in most other rat strains, and its background incidence has increased significantly over time (Caldwell, 1999). Earlier reports inform incidence of 30.5 % for males and 20.5% for females (Chandra and Frith, 1992) while later ones report an incidence in males around 50 % and in females around 30 % (Caldwell, 1999). A large variation from study to study has been reported (Caldwell, 1999). Historical incidence in 2-year feed studies by NTP (with controls given the NTP-2000 diet as in the study with Benzophenone) was in a range of 30-68 % for males and in a range of 12-38 % in females (NTP, 2006). These data indicate also that the incidence is higher in males than in females.

The evaluating MS noted that no NOAEL could be identified for MNCL in both sexes in the NTP study in F-344 rats. In males there was no dose response for incidences of MNCL. Incidence in the male high-dose group was comparable to that in the control group, and both incidences were within the historical range reported by the authors of the study. Only incidences in the low- and middle-dose males were statistically significantly higher than in the concurrent control and exceeded the historical incidence. In treated females a statistically significantly

increase in the incidence of MNCL was recorded only in the middle-dose group but the incidence in the high-dose group was not different from that in the middle-dose group from the biological point of view (60 % at the middle dose versus 58 % in the high-dose). The lack of a statistically significant difference in the incidence of MNCL between the female low-dose group and the control suggest that the low-dose could be a NOAEL corresponding to 15 mg Benzophenone/kg bw/day.

According to the evaluating MS the relation to treatment of MNCL in both sexes was suggested by the fact that incidences in males from the low and middle–dose groups and in females in all treated groups exceeded the historical incidences reported by the authors of the study. However, the fact that a spontaneous incidence of this neoplasm has significantly increased over time complicates retrospective data interpretation and may question the relation to treatment in this study.

Finally, it has to be noted that MNCL as F-344 rat strain specific tumour is of questionable relevance for humans. It has been proposed that substances which increase MNCL frequency are appropriately classified as category 3 under the International Agency for Research on Cancer (IARC) classification scheme (not classifiable as to its carcinogenicity to humans) (Caldwell 1999).

The lower than in the control group incidences of mammary gland fibroadenoma in female middle-and high-groups and the decreased incidences of keratoacanthoma are not considered by the evaluating MS to be related to the treatment with Benzophenone in accordance with the evaluation of the authors of the study (NTP 2006).

In relation to histiocytic sarcoma of haematopoietic system in female F-344 rats the evaluating MS noted that this is a rare tumour as indicated by the historical incidence reported in the literature (1.6 % and 1.2 % in male and female F-344 rats, respectively; Ogasawara *et al*., 1993).This malignant neoplasm was not observed in the historical feed study of control rats given NTP-2000 diet and its incidence for all routes in 2-year rat studies was in a range of (0.08 %) 0-2 % (NTP, 2006).

The relation to treatment of histiocytic sarcoma in female rats in the middle- and high-dose groups was supported by the facts that this tumour is rare. The tumour form is also rare in mice (1.4 % in historical feed study). Histiocytic sarcomas were recorded in female mice from the middle and the high-dose groups in multiple organs in the 2-year assay with Benzophenone (NTP, 2006). With regard to this tumour in female rats a NOAEL could be the low dose corresponding to 30 mg Benzophenone/kg bw/day according to the evaluating MS. The lowest BMDL with regard to this malignant neoplasm was 62.5 mg Benzophenone/kg bw/day as calculated by the evaluating MS.

7.9.6.3. Carcinogenicity: dermal

Stenbäck and Shubik (1974) investigated in female Swiss mice potential carcinogenic effect of several compounds following a topical administration. Benzophenone was administrated topically (on 1-inch square of the dorsal skin between the flanks, which was shaved regularly) in concentrations of 5, 25, and 50 % in acetone in a total volume of 0.2 ml, twice a week for a period of up to 110 weeks. A vehicle control group received the same treatment with acetone and a positive control was treated in the same manner with dimethylbenzanthracene (DMBA). The number of animals in the test groups, in the vehicle and positive control groups was 50/group. The additional untreated control group consisted of 150 animals. All mice in Benzophenone treated groups were dead/killed by week 110, while there were still few survivors in the acetone group (3/50) or in the untreated control group (9/150).

The total number of tumour bearing mice was 26/50 (52 %), 16/50 (32 %) and 14/50 (28 %) in the low, middle and high-dose Benzophenone treated groups. For comparison, the total

number of tumour bearing animals in the vehicle control group was 22/50 (44 %), in the positive control group 39/50 (78 %), and in the untreated control group 64/150 (42 %).

The total number of tumours and type of tumours are presented in Table 11 below. The data presented are extracted from Table 1 in the original paper.

Table 11 Overview of the total number of tumours and type of tumours:

Group	Total no of tumour	Lym-phomas	Lung adenomas	Liver haeman-gionas	Thy-momas	Skin tumours	Others
Benzophenone							
5 %	38	15	3	1	1	2	16
25 %	22	11	3	1	1	1	5
50 %	18	6	6	2	-	-	4
Vehicle: Acetone	31	12	9	2	-	2	6
Positive control DMBA	86	6	4	1	-	76	-
Untreated control	72	26	17	4	6	3	16

Benzophenone treated animals had a small number of skin tumours. The skin tumours in the 5 % treated group were squamous cell papillomas (benign tumours) on dorsal skin. In the 25 % treated group the tumour was a squamous cell carcinoma on the lip.

In Benzophenone treated groups the highest number of animals bearing tumours was in the low-dose group and it was not statistically significantly higher than in the positive control group (26/50 versus 39/50; p>0.05, Fisher's exact test performed by the evaluators). There was no dose response in total number of tumours, lymphomas, thymomas, skin tumours and other tumours. The numbers of liver adenomas and haemangionas were low and not higher than in the vehicle control group or in the untreated control group.

The total number of tumour bearing animals, the total number of tumours, and other tumour data from the table above do not indicate a carcinogenic potential of Benzophenone in mice following a topical administration under conditions of this bioassay.

It has been noted by the evaluating MS that this study was published in 1974, and as such had not been performed according to Good Laboratory Practice (GLP) and Organisation for Economic Co-operation and Development (OECD) guidelines. However, according to the evaluating MS, the fact that the study is old and was not performed according to current standards should not *per se* disqualify the study from being included in the risk assessment of Benzophenone. As long as the design of any such study and the reporting of the data are considered appropriate with regard to the endpoint of interest, the evaluating MS is of the opinion that the study should be included in the evaluation as a part of supportive evidence.

Stenbäck (1977) investigated a potential carcinogenicity of several compounds following epicutaneous administration in New Zealand rabbits. Three experimental groups (5 rabbis/groups, both sexes, a number of females and males/group not informed) were treated with 0.2 ml Benzophenone in concentration of 5, 25 and 50 % in a solvent. Benzophenone solutions were applied to interior left ear. A positive control (15 rabbits/ groups, both sexes, a

number of females and males/group not informed) was treated in the same manner with DMBA (a dosing volume of 0.2 ml) twice a week. The treatment continued until the animals died spontaneously. Two untreated control groups (4 or 5 rabbits/group, a number of females and males in the group not informed) were also included. The evaluating MS noted that it was not clear from the description of the study whether the solvent was acetone or methanol and no data on the vehicle control group were included in the paper. The survival at week 160 was 1, 3 and 2 rabbits in the low, middle, and high-dose Benzophenone groups, and 3 in the untreated control group with an initial number of animals of 5. In another control group (4 rabbits in the start) no survivals were present in week 120. According to the author of the study, the positive control was terminated in week 50 for morphological analyses. No tumours were recorded in the Benzophenone treated group and in the untreated control groups. In the positive control group 12 tumours were recorded.

According to evaluating MS the results could indicate that the treatment with Benzophenone had no effect in survival. The lack of tumours in the Benzophenone treated groups may indicate the lack of carcinogenicity of topically applied Benzophenone in accordance with the results of a study in mice (Stenbäck and Shubik, 1974) and with the presumed non-genotoxic mode of action of Benzophenone (see section 7.9.5 Mutagenicity). However, as indicated by the author of the study, the total dose might be too small due to volume and frequency of applications, duration of the experiment, life span of the animal and solubility of the compound.

Considering shortcomings in the design of the study indicated by the author, the low number of animals per group and lack of the data on the vehicle control it is concluded by the evaluating MS that this study neither prove or disprove the lack of carcinogenic potential of Benzophenone following a topical administration. It has also to be noted that this study was published in 1977, and as such had not been performed according to GLP and OECD guidelines.

7.9.6.4. Summary and discussion of carcinogenicity

The data from long-term studies with Benzophenone via dietary exposure in mice and rats conducted by (NTP, 2006) pointed to liver and kidney as target organs of Benzophenone chronic toxicity. Neoplastic responses were recorded in the liver (mouse), the kidney (rat), and in hematopoietic system (rat and mouse), and some of the findings are shown in table 12 and 13 below.

Furthermore, statistically significant and/or biologically noteworthy non-neoplastic changes were found in the liver and kidneys in mice and rats, in the spleen, nose and testes in mice and in the kidney, liver, and thyroid gland in rats. These findings are shown in table 14 and 15.

Table 12 NTP, mice: Neoplastic changes (%)

Findings / Dose	Control	312 ppm	625 ppm	1250 ppm
Male hepatocellular adenoma (multiple)	4	16 *	16 *	24 **
Carcinomas	16	10	12	12
Hepatoblastoma	0	2	2	6

Combined (male) hepatocellular carc and hepatoblastoma	16	12	14	18
Histiocytic sarcoma (females)#	0	0	10	6
Female hepatocellular adenoma	2	2	6	6

*Significantly different (P≤0.05) from the control group
**P ≤0.01
Historical control: 0.44% (range: 0-2%)

Table 13 NTP, rats: Neoplastic changes (%)

Findings / Dose	Control	312 ppm	625 ppm	1250 ppm
Renal tubule adenoma/ carcinoma Male	4/0	4/1	14/0	16/0
MNCL Males Females	54 38	82 50	78 60	48 58
Histocytic sarcomas (females) ##	0	0	2	4

Historical control: 0.08 % (range 0-2%)

Table 14 NTP, mice: Non-neoplastisc changes (%) (312, 625, 1250ppm / 35-40,70-80,105-150 mg/kg bw/d)

Findings / Dose	Control	312 ppm	625 ppm	1250 ppm
Female Hypertrophy of hepatocytes	0	58**	88**	74**
Male Multinucleated hepatocytes	0	82**	94**	96**
Active chronic inflammation Male Female	66 88	94** 80	88** 82	84* 72*

Nephropathy				
Male	98	96	100	100
Female	42	66**	62*	60*
Spleen hyperplasia				
Males	34	62**	68**	64**
Females	48	72**	74**	44
Hematopoietic cell proliferation	32	70**	64**	54*

*Significantly different (P≤0.05) from the control group by the Poly-3 test
**P ≤0.01

Table 15 NTP, rats: Non neoplastic changes (%) (312, 625, 1250ppm / 15, 30 ,60 mg/kg bw/d)

Findings / Dose	Control	312 ppm	625 ppm	1250 ppm
Hyperplasia of transitional epithelium renal pelvis				
Males	2	22**	58**	68**
Female	2	4	4	8
Renal tubule hyperplasia				
Males	6	22*	60**	80**
Females	2	16*	20**	14*
Centribular hypertrophy of hepatocytes			62**	
			60**	38**
Males	0	34**		66**
Females	0	54**		
Cystic degeneration				
Males	16	22	40**	30*
Active chronic				

inflammation				
Males	44	42	70**	66*
Females	92	76*	58**	60**
Bile duct hyperplasia				
Females	20	70**	78**	80**
C-cell hyperplasia thyroid gland				
Males	34	16*	16*	10*
Females	68	22**	26**	16**

*Significantly different (P≤0.05) from the control group by the Poly-3 test
**P ≤0.01

Two studies using dermal exposure were also included in the evaluation (Stenbäck and Shubik, 1974 and Stenbäck, 1977).

Based on the available data the evaluating MS concludes that there is no concern with respect to genotoxicity of Benzophenone. With regard to non-genotoxic carcinogens there is consensus in the scientific community that a threshold for their carcinogenic effect exists.

Under the conditions of the NTP 2-year studies in mice and rats, the reports concluded that there was *some evidence of carcinogenic activity* of Benzophenone in male F344/N rats based on increased incidences of renal tubule adenoma. Mononuclear cell leukemia (MNCL) in male F344/N rats may have been related to Benzophenone exposure. There was *equivocal evidence of carcinogenic activity* of Benzophenone in female F344/N rats based on the marginally increased incidences of mononuclear cell leukemia and histiocytic sarcoma. There was *some evidence of carcinogenic activity* of Benzophenone in male B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was *some evidence of carcinogenic activity* of Benzophenone in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcoma; the incidences of hepatocellular adenoma in female B6C3F$_1$ mice may have been related to Benzophenone exposure.

The liver tumours of significance were recorded in male B6C3F$_1$ mice: hepatocellular adenoma, hepatoblastoma and carcinoma. For multiple hepatocellular adenoma the low-dose corresponding to 40 mg/kg bw/day was a LOAEL and for total adenoma (included multiple ones) this dose was a NOAEL. In relation to combined incidences of adenoma, hepatoblastoma and carcinoma a NOAEL was the middle dose corresponding to 80 mg/kg bw/day.

The kidney tumours of significance were renal tubule adenomas in F-344 male rats. The NOAEL for this benign neoplasm was the middle dose corresponding to 30 mg/kg bw/day. The lowest Benchmark dose low (BMDL) calculated by the evaluators for renal tubule adenoma incidences in male rats was 16.6 mg Benzophenone/kg bw /day.

In relation to MNCL it was noted that an increased incidence of MNCL in long-term studies in F-344 rats is considered of limited relevance for humans, re. 6.9.6.2.3.

Histiocytic sarcoma of the hematopoietic system was recorded in female B6C3F$_1$ mice and female F-344 rats. The tumour type is rare in both species. The evaluating MS concludes that based on the incidences of this sarcoma there is some evidence of carcinogenic activity of Benzophenone.

Overall, based on the available evidence, the evaluating MS concludes that Benzophenone meets the criteria for classification as carcinogenic in category 2.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not evaluated.

Results from the reproductive toxicity studies, which are relevant for the evaluation of possible endocrine disrupting effects of Benzophenone, are presented and discussed in section 7.10.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Available and appropriate data has been considered when identifying the DNEL, and the study or studies giving rise to the highest concern has been used to establish the DNEL.

The evaluating MS has examined EFSA's Scientific Opinion, Toxicological evaluation of Benzophenone, in which the Scientific Opinion of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF) has noted that Benzophenone causes kidney adenoma in rat, associated with a spectrum of responses including hyperplasia and nephropathy at the lowest dose level of 15 mg/kg bw/day in a chronic carcinogenicity study (NTP, 2006). The Panel considered that the non-neoplastic kidney effects observed in the chronic assay were adverse. Benchmark dose (BMD) analyses were applied for the non-neoplastic kidney effects in male rats, and the lower 95 % confidence limits of the benchmark dose for a 10 % effect (BMDL 10) were calculated to be 3.1 to 7.4 mg/kg bw/day. The models used in the analysis were consistent, and passed statistical validation. The Panel decided that the BMDL 10 value of 3.1 mg/kg bw/day was the most appropriate departure point for derivation of the TDI. By applying an uncertainty factor of 100, a TDI of 0.03 mg/kg bw/day is derived.

For the purpose of providing an urgent advice to the risk managers, the EFSA in its statement considered the liver hypertrophy seen in the two generation reproductive toxicity study (Hoshino *et al.*, 2005) with Benzophenone as adverse effects. Thus, EFSA derived a LOAEL of 6 mg Benzophenone/kg bw/day from this study.

However, a LOAEL of 15 mg/kg bw/day derived from a 2-year carcinogenicity study is used as basis for the DNEL. The evaluating MS requested in a draft decision a justification of not using the lowest value for NOAEL or LOAEL, and subsequently as a justification for this it was stated that the use of the LOAEL of ca. 6 mg/kg bw/day derived from the two generation reproductive toxicity study may have some disadvantages, as the liver hypertrophy seen in rats was not correlated with an increase in liver weights and may therefore be an adaptive but not a true adverse response. This was also described by EFSA (EFSA, 2009). The evaluating MS agreed to this.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

The substance evaluation has focussed on the end-points of carcinogenicity and endocrine disruption. No other health hazard end-points were evaluated. Based on evaluation of the available data, the evaluating MS concludes that Benzophenone is tumourigenic in the liver, the kidney and the haematopotic system in animals. The evidence supports a non-genotoxic mode of action, thus a threshold for the carcinogenic effect can be set. The evaluating Member State evaluates that Benzophenone should be classified as carc. 2; H351. Acording to the CLP regulation, article 36(c), a proposal for classification for carcinogenicity will be prepared as a follow-up to this substance evaluation.

7.10. Assessment of endocrine disrupting (ED) properties

Endocrine disrupting properties were evaluated as a concern for human health and the environment.
Based on examinations of studies published in the open literature, the evaluating MS considers that endocrine disruptive effects (estrogenic and thyroid disruptive potential) of Benzophenone and its metabolites/degradation products cannot be fully excluded, as described in the following.

7.10.1. Endocrine disruption - Human health and the Environment

7.10.1.1. Estrogenicity and anti-androgenecity

Based on examinations of studies published in the open literature, there are some remaining unclarified indications regarding potential estrogenic properties of hydroxylated metabolites and environmental degradation products of Benzophenone. Relevant studies investigating estrogenic properties are listed below.

Table 16 Overview of investigations of estrogenic activity in vitro (BP: Benzophenone)

Description of study	Observation of estrogenic effects?	Tested substance
MCF-7 cell proliferation (Nagakawa *et al*., 2000)	No	BP
ER activation (Yamasaki *et al*., 2002)	No	BP
ER binding (Hayashi *et al*., 2006)	No	BP
MCF-7 reporter assay (Suzuki *et al*., 2005)	No	BP
MCF-7 cell proliferation (Nagakawa *et al*., 2000)	Yes	4-OH-BP
MCF-7 reporter assay (Suzuki *et al*., 2005)	Yes	3-OH-BP and 4-OH-BP
ER activation (Yamasaki *et al*., 2002)	Yes	3-OH-BP and 4-OH-BP
ER binding (Hayashi *et al*., 2006)	Yes	3-OH-BP and 4-OH-BP

Table 17 Overview of investigations estrogenic activity *in vivo* (BP: Benzophenone)

Description of study	Indication of estrogenic effects?	Tested substance
Subchronic repeated dose toxicity, OECD 408. 14 weeks of dosing in diet in B6C3F1 mice (NTP, 2000).	No No effect on sperm motility or vaginal cytology in any dose group	BP (First pass metabolism possible)
Subchronic 90 day (28 day) study in rats, dosed in diet with 20, 100 and 500 mg/kg/day. After 28 days all mid and high dose animals and 50 % of the low dose animals were sacrificed (Burdock *et al*., 1991).	No No effects on weight of testes, epididymis or ovaries and no effect on histopathology of mammay, ovary, uterus, testes, seminal vescicle or prostate	BP (First pass metabolism possible)
Subchronic repeated dose toxicity, 14 weeks of dosing in diet, in Fisher 344/N rats. OECD 408. (NTP, 2000)	No No effect on sperm motility or vaginal cytology in any dose group	BP (First pass metabolism possible)

Description of study	Indication of estrogenic effects?	Tested substance
Uterotrophic, oral, OVX SD rats (Nagakawa & Tayama, 2002)	Yes NOEL 100 mg/kg/d LOEL 400 mg/kg/d	BP (First pass metabolism possible)
Uterotrophic, intraperitoneal, OVX F344 rats (Suzuki *et al.*, 2005)	Yes NOEL 100 mg/kg/d LOEL 300 mg/kg/d	BP (First pass metabolism possible)
Uterotrophic, subcutaneous, juvenile SD rats (Nakagawa & Tayama, 2001)	No NOEL 400 mg/kg/d	BP
Uterotrophic, subcutaneous, juvenile SD rats (Yamasaki *et al.*, 2002)	No NOEL 200 mg/kg/d	BP
Uterotrophic, subcutaneous, juvenile SD rats (Hayashi *et al.*, 2006)	No NOEL 1000 mg/kg/d	BP
Uterotrophic, subcutaneous, juvenile SD rats (Nagakawa & Tayama, 2001)	Yes LOEL 100 mg/kg/d	4-OH-BP
Uterotrophic, subcutaneous, juvenile SD rats (Yamasaki *et al.*, 2002)	Yes NOEL 40 mg/kg/d LOEL 200 mg/kg/d	4-OH-BP
Uterotrophic, subcutaneus, juvenile SD rats (Hayashi *et al.*, 2006)	Yes LOEL 150 mg/kg/d NOEL 100 mg/kg	4-OH-BP
Uterotrophic, subcutaneous, juvenile SD rats (Hayashi et al., 2006)	Yes LOEL 500 mg/kg/d NOEL 250 mg/kg	3-OH-BP
Two-generation reproductive toxicity study, OECD 416, diet, SD rats (Hoshino *et al.*, 2005)	No NOAEL 120 mg/kg/d during gestation, 320 mg/kg/d during lactation. No effect on anogenital distance in F1 and F2 offspring, timing of sexual maturation in F1 offspring, weigths and histopathology of testes, epididymides, prostates, seminal vesicles, ovaries and uterus in F1 parental animals, and levels of testosterone, FSH, LH and estradiol, estrous cyclyicity	BP (First pass metabolism possible)

Description of study	Indication of estrogenic effects?	Tested substance
	and semen quality in F0 and F1 animals.	

As described in the toxicokinetics section (section 7.9.1), Benzophenone was in rats found to be converted to 4-OH-Benzophenone, its sulphate conjugate and sulfhydrol. Further, 4-OH-Benzophenone and 3-OH-benzophonone have been observed to be formed from Benzophenone by photolytic degradation in water (section 7.7.1).

Studies investigating estrogenic and anti-androgenic effects of Benzophenone and its metabolites/degradation products are summarised below.

7.10.1.1.1. Estrogenicity and anti-androgenicity *in vitro* and *in silico*

Benzophenone and some hydroxylated metabolites/degradation products have been investigated in several *in vitro* studies for endocrine disrupting properties. Benzophenone itself showed no effect on MCF-7 cell proliferation (Nakagawa *et al*., 2000), no effect on ER-mediated transcriptional activation in a reporter gene assay in human cervical carcinoma cell (Yamasaki *et al*., 2002), no binding affinity to the ER (Hayashi *et al*., 2006) and no estrogenic activity in a luciferase reporter assay in MCF7-cells (Suzuki *et al*., 2005).

However, 4-OH-BP showed weak estrogenic activity on MCF-7 proliferation (Nakagawa *et al*., 2002), and both 3-OH-BP and 4-OH-BP increased estrogenic activity in a luciferase reporter assay in MCF7-cells (Suzuki *et al*., 2005), activated ER mediated transcription (Yamasaki *et al*., 2002) and showed binding affinity to the ER (Hayashi *et al*., 2006).

Suzuki *et al*. (2005) furthermore found that both 3-OH-BP and 4-OH-BP acted as weak anti-androgens in an ARE-luciferase reporter assay.

Furthermore, four of the predicted microbial metabolites (using the OECD Toolbox v. 3.4 Microbial Metabolism Simulator) had alerts as very strong estrogenic binders *in vitro* (i.e. (2,3-dihydroxy-phenyl)-phenyl-methanone and (3,4-dihydroxy-phenyl)-phenyl-methanone) and two were predicted to be strong estrogenic binders *in vitro* (i.e. (2,3-dihydroxy-phenyl)-(3,4-dihydroxy-phenyl)-methanone and Bis(2,3-dihydroxyphenyl)-methanone. Three of these (all except Bis(2,3-dihydroxyphenyl)-methanone) were also predicted to have *in vitro* estrogenic receptor binding/activities and/or antiandrogenic receptor binding/activities by the publicly available Danish QSAR Database[4] (see Annex 1). (3,4-dihydroxy-phenyl)-phenyl-methanone was also predicted by EAWAG Biocatalysis/ Biodegradation Database as the first biotic degradation step. Thus there are strong indications that Benzophenone can be degraded in water into ring-hydroxylated derivatives that have estrogenic and/or anti-androgenic activity *in vitro*.

Overall, based on the results from the performed *in vitro* assays and available *in silico* data, it seems that even though Benzophenone itself has shown no estrogenic potential, several metabolites and degradation products seem to have an estrogen potential. Inhibition of androgen receptor activation is also a potential mechanism of action, as observed *in vitro*.

7.10.1.1.2. Estrogenicity and anti-androgenicity *in vivo*

In the NTP 14-week studies, the effects of Benzophenone on reproduction were assessed by evaluation of testicular and spermatozoal parameters and by characterization of the estrous

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[4] http://qsar.food.dtu.dk/

cycle. Sperm motility and vaginal cytology evaluations were performed on rats in the 0, 1,250, 2,500, and 5,000 ppm groups and on mice in the 0, 2,500, 5,000, and 10,000 ppm groups at the end of the studies. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various estrous stages. The testis and epididymis weights of male mice in the 10,000 ppm group were significantly less than those of the controls. There were no significant differences in sperm motility or vaginal cytology parameters between exposed and control males or females. These results indicate that Benzophenone does not induce antiandrogenic or estrogenic effects in adult animals. However, assessment of reproductive endpoints like estrous cyclicity and semen parameters in rodents treated with a compound for 90 days during adulthood is not a sufficiently sensitive measure of the potential for endocrine disruption.

In order to assess the estrogenic properties *in vivo* further, some uterotrophic assays have been performed and in addition a number of endpoints sensitive to endocrine disruption has been investigated in a two-generation reproductive toxicity study (OECD 416). The studies are referred below.

In an uterotrophic assay Benzophenone was given orally at doses of 100 and 400 mg/kg/day for 3 days to ovariectomized (OVX) SD rat (Nakagawa & Tayama 2002), and significantly increased uterine weights were seen in the highest dose group. Furthermore, increased luminal epithelium height, increased thickness of the stromal layer in the uterus and histological changes in vaginal cornification were seen, indicating an estrogenic effect. In another study intraperitoneal doses of 100 or 300 mg/kg/day were given to OVX F344 rats for three days (Suzuki *et al*., 2005), and here the highest dose also significantly increased uterine weights. This indicates that higher doses of Benzophenone can yield an estrogenic response, when using oral or intraperitoneal dosing.

In the first uterotrophic assay performed with Benzophenone, juvenile rats where subcutaneously (SC) injected for three days at a dose of 400 mg /kg bw/day, which did not affect the uterine weight (Nakagawa & Tayama, 2001). Similar results were observed by Yamasaki *et al*., (2002). In an uterotrophic assay using immature rats, there was no uterine response after SC injection of 2, 20 or 200 mg/kg for 3 days. A Benzophenone dose of 1000 mg/kg/day injected SC to immature SD-rats also did not increase uterine weight in a study by Hayashi *et al*. (2006).

However, the Benzophenone metabolite 4-OH-BP has been shown to elicit an estrogenic effect in several uterotrophic assays. Nakagawa & Tayama (2001) found that 4-OH-BP doses of 100, 200 and 400 mg/kg SC injected, significantly increased uterine weights. Increased uterine weights were also seen at subcutaneous 4-OH-BP doses of 200 and 800 mg/kg in an uterotrophic assay using immature rats by Yamasaki *et al*. (2002), whereas no significant effect was seen at 40 mg/kg. Similarly, another uterotrophic assay showed increased uterine weights in immature SD-rats treated with 4-OH-BP and 3-OH-BP. 4-OH-BP was the more potent of the two metabolites, as doses of 150 mg/kg and above significantly increased uterine weights, whereas only doses of 500 and above of 3-OH-BP had a significant effect (Hayashi *et al*., 2006). It is possible that the observed uterotrophic effects seen after oral or intreperitonal dosing of Benzophenone were caused by its metabolism to hydroxylated forms, as Benzophenone seems to acquire estrogenic activity *in vitro* after ring- hydroxylation (Nakagawa & Tayama, 2002). In contrast, in the uterotrophic assays where Benzophenone was injected SC, it is plausible that no such hydroxylation occurred, due to lack of first pass metabolism, and hence no estrogenic effect was observed.

The reproductive toxicity and endocrine disrupting potential of Benzophenone have also been assessed in a two-generation reproductive toxicity study (OECD 416) (Hoshino *et al*., 2005). The dose levels of Benzophenone were 0, 100, 450 and 2000 ppm given in the diet, using SD rats. As described by Hoshino *et al*. (2005) results from a previous range-finding study included reduction in body weight and inhibition of food consumption in the 20000 ppm group,

and all these animals underwent moribund sacrifice. The lower doses caused decreased body weights in the 6000 ppm group, and lower food consumption in the females from the 2000 and 6000 ppm groups. Elevated absolute and relative hepatic and renal weights were seen in both males and females from the 600 ppm and higher. Moreover, with regard to effects on the reproductive organs, the 2000 and 6000 ppm groups showed elevated values for absolute and relative testicular weights. The 6000 ppm group demonstrated low values for absolute and relative epididymal weights. Based on these results, the highest dose for the definitive study was set at 2000 ppm. The intermediate and lowest doses were calculated by dividing by a common ratio of about 4.5, and set as 450 and 100 ppm, respectively.

When calculated as mg/kg bw/day this lowest dose (100 ppm) corresponded to 6-15 mg/kg, the middle dose (450 ppm) to 25-70 mg/kg and the highest dose (2000 ppm) to 120-320 mg/kg with the rats receiving the lowest doses within this range during the gestation period, and the highest ones during lactation. The mean dose levels in females throughout the study were 8, 39 and 172 mg/kg/day in the three dose groups respectively. Endpoints of particular relevance for endocrine disruption were evaluated in the two-generation study: anogenital distance F1 and F2 offspring, timing of sexual maturation in F1 offspring, weights and histopathological evaluation of testes, epididymis, prostates, seminal vesicles, ovaries and uterus in F1 parental animals, and levels of testosterone, FSH, LH and estradiol, estrous cyclicity and semen quality in F0 and F1 animals. At the tested dose levels, none of these endpoints were significantly affected by Benzophenone. In the highest dose group, both male and female offspring showed significantly reduced body weights at the time of weaning, continuing throughout the rest of the study. The two highest doses also caused increased liver and kidney weights in F0 and F1 adult males and females and histopathological changes in the kidneys. The effects on body weight, liver and kidney indicate that some systemic toxicity is induced at the highest dose level, and testing in higher doses is therefore not possible in this study design. At the tested doses Benzophenone was not a developmental and reproductive toxicant and it did not induce endocrine disruptive effects (Hoshino *et al*., 2005).

The estrogenic potential of Benzophenone itself has also been investigated in an *in vivo* bioassay in zebrafish by Brion *et al*. (2012). In this study, the potential of natural or synthetic steroids or ubiquitous environmental contaminants to alter cyp19a1b-driven GFP (green fluorescent protein) expression in RGCs (radial glial cells) of developing zebrafish was investigated. Fertilized cyp19a1b-GFP transgenic zebrafish eggs were exposed to chemicals or to solvent control (DMSO; 0.01% v/v) under semi-static conditions from 0 to 5 days post-fertilization. At the end of exposure, the 5 days old post-fertilization zebrafish were processed for cyp19a1b GFP expression by polymerase chain reaction (PCR) or for fluorescence measurement by image analysis. For the test substance Benzophenone, no induction of GFP expression was found in these experiments and thus indicating that Benzophenone does not act as an estrogen mimic in this experimental set-up.

7.10.1.1.3. Discussion regarding estrogenicity and anti-androgenicity - human health

The concern about anti-androgenic acitivity is raised by anti-androgenic activity *in vitro*, since the metabolites 3-OH-BP and 4-OH-BP acted as weak anti-androgens in an ARE-luciferase reporter assay (Suzuki *et al*., 2005). *In vivo*, the conducted two-generation reproductive toxicity study did not show significant effects or tendencies towards altered anogenital distance, weights and histology of reproductive organs, serum hormone levels, timing of sexual maturation or semen quality in developmentally exposed offspring. As described below, there is some remaining uncertainty regarding whether high enough doses were used in this study. However, since some systemic toxicity was observed in the highest dose group, it seems not possible to use higher doses in a generational study design.

The concern about estrogenic activity is raised by the estrogenic activity *in vitro* by the hydroxylated metabolites of Benzophenone combined with estrogenic activity observed in uterotrophic assays using oral or interperitoneal dosing of Benzophenone (allowing first pass metabolism) as well as in uterotrophic assays with the metabolites 3-OH-BP and 4-OH-BP.

In spite of the fact that several studies on Benzophenone have been conducted, estrogenic effects of Benzophenone cannot be ruled out. Even though the two-generation reproductive toxicity study did not show significant effects or tendencies towards altered anogenital distance, weights and histology of reproductive organs, serum hormone levels, estrous cyclicity, timing of sexual maturation or semen quality in developmentally exposed offspring, and that a large group size was used and many endpoints relevant for endocrine disruption were investigated in the study, some doubts on the estrogenic effects still remain.

The reasons for the remaining uncertainties are: The parameters which are more sensitive to estrogenic compounds (e.g. mammary gland whole mounts in young female offspring and quantitative assessment of follicular maturation in adult female offspring) were not investigated. Furthermore, the highest dose level used in the 2-generation reproductive toxicity study (Hoshino *et al*., 2005) (for the females 120 mg/kg/d during gestation) is suspected to be too low and not sufficient to induce estrogenic (and/or anti-androgenic) effects, since in the uterotrophic studies performed with Benzophenone by oral or interperitoneal dosing, the NOELs are 100 mg/kg/d, and thus very close to the highest dose in the 2-generation reproductive toxicity study, whereas the LOELs are 300 and 400 mg/kg/d, respectively. However, since effects indicating systemic toxicity (on body weight, liver and kidney) were observed in the highest dose group in the 2-generation reproductive toxicity study, it will be difficult to test this further in a similar type of study.

The evaluating MS has during substance evaluation considered to request the following studies in order to clarify the concern for estrogenic effects :

- An Extended One Generation Reproductive Toxicity Study (EOGRTS) (OECD 443). This is the higher tier test guideline study, which currently investigate endocrine disruptive properties to the greatest extent. The older 2-generation reproductive toxicity study available on Benzophenone does not include investigation of endpoints most sensitive to an estrogenic mode of action, i.e. evaluation of mammary gland whole mounts in young female offspring and quantitative assessment of follicular maturation in adult female offspring. However, these endpoints are not mandatory in the EOGRTS (OECD TG 443), and would have to be included as additional endpoints based on the raised concern. Furthermore, the effects observed on body weight, liver and kidney in the highest group in the 2-generation reproductive toxicity study, indicate that some systemic toxicity was induced at the highest dose level, and testing in higher doses is therefore not feasible in this study design. It is therefore doubtful that an EOGRTS will be possible to conduct at higher dose levels, which may be suspected to induce estrogenic (and/or anti-androgenic) effects. In conclusion, it currently does not seem justified – both scientifically, economically and for animal welfare reasons - to request this extensive study.

- Investigation of estrogen related endpoints in the 28-days Repeated Dose Toxicity Test (EU B.7 (OECD 407)).
  However, the ability of this study to detect weak estrogenic substances is doubted, since the report on the validation of the updated OECD 407 (OECD Series on Testing and Assessment, No. 59, 2006) concluded that this lastly updated test guideline seemed only to be able to identify strong and moderate estrogenic substances (based on validations with nonylphenol and genistein). Therefore, it is questionable that this test method will be able to identify estrogenicity caused by Benzophenone *in vivo*, because Benzophenone is suspected for only being a weak *in vivo* estrogenic substance.

Based on the above considerations, it is the view of the evaluating MS that even though there is some residual concern for estrogenic effects in mammals, there is no obvious way to clarify this, both due to the large number of *in vivo* studies already conducted (including an older 2-generation reproductive toxicity study which included a number of ED-relevant endpoints), and due to the fact that currently there are no reliable short term test methods to easily and adequately investigate this further in rodents.

7.10.1.1.4. Discussion regarding estrogenicity - Environment

Estrogen receptors are conserved across taxonomic classes, and estrogenic effects are relevant to both humans and to vertebrate wildlife species. The observations *in vitro* and in rodents *in vivo* therefore also give rise to a concern for estrogenic effects of the metabolites of Benzophenone in non-mammalian vertebrate species relevant for the environment.

The evaluating MS recognizes that Benzophenone itself does not seem to have estrogenic ED properties (as observed *in vitro*, in the uterotrophic assay and in the zebrafish bioassay). However, as described in the section about environmental fate properties of Benzophenone (section 7.7), formation of estrogenic active substances are likely when Benzophenone is degraded in the environment and also when metabolised in animal wildlife, since the known or likely degradation products and metabolites also include estrogenic active metabolites. Further, estrogenic responses have been observed in some rodent studies where first pass metabolism occurs, supporting the concern for endocrine disruption related to the estrogenic metabolites of Benzophenone. The evaluating MS is also aware of the fact that the estrogenic metabolite 4-OH-BP only seems to be a minor metabolite in rats. However, there may be differences in the metabolic transformation between species, because of the formation of hydroxylated metabolites / transformation products of Benzophenone form in fish or by environmental degradation processes in the aquatic environment.

As Benzophenone is rapidly and ultimately degraded in the environment the degradation products of Benzophenone are implicitly also rapidly fully and quickly degraded in the environment under most environmental conditions. Additionally, due to the hydroxylation taking place under both environmental degradation and when Benzophenone is metabolised in animal wildlife, the degradation products/metabolites of Benzophenone are less hydrophobic / lipophilic and are thus not expected to bioaccumulate but to be excreted rapidly by animal wildlife. This is also supported by the low BCF value of Benzophenone measured in fish. All together, there is some residual concern that the degradation products and/or metabolites of Benzophenone could cause estrogenic activity resulting in adverse effects in aquatic wildlife such as fish. However, the degree of concern is low due to the fast transformation of the suspected degradation products/metabolites and due to their low bioaccumulation potential. The relative extent of formation of estrogenic degradation products/metabolites that occur under environmental degradation processes and by internal metabolism in various species is also only known in a very fragmented way (c.f. the sections above about toxiocokinetics in rat and environmental fate properties). It is, however, likely that the relative concentration of such degradation products/metabolites would not be high, that they will only occur transiently as they will be rapidly further transformed, and that they are unlikely to bioaccumulate to any significant extent.

7.10.1.1.5. Estrogenic/antiandrogenic effects - conclusion

In conclusion, for estrogenic potential, the concern is raised by estrogenic activity of Benzophenone metabolites and environmental degradation products *in vitro* and *in vivo* (in the uterotrophic assay).

The evaluating MS finds that currrently there is no straightforward way to follow up on the concern for human health in respect to the oestrogenic activity of Benzophenone, since a number of long-term rodent studies are already available, including an older 2-generation reproductive toxicity study, which did not detect effects in some endpoints sensitive to endocrine disruption (i.e. anogenital distance F1 and F2 offspring, timing of sexual maturation in F1 offspring, weights and histopathological evaluation of testes, epididymis, prostates, seminal vesicles, ovaries and uterus in F1 parental animals, and levels of testosterone, FSH, LH and estradiol, estrous cyclicity and semen quality in F0 and F1 animals). It is acknowledged that the most sensitive endpoints currently known for detection of estrogenic effects were not included in this 2-generation reproductive toxicity study (e.g. evaluation of mammary gland whole mounts in young female offspring and quantitative assessment of follicular maturation in

adult female offspring). However, even though the doses used in the 2-generation study may have been too low to induce estrogenic effects (when compared to the doses inducing estrogenic effects in the uterotrophic assays), the highest dose induced some systemic toxicity, and testing in higher doses is therefore evaluated not to be appropriate in a similar test design. Further, currently no reliable short term test methods exist which could be requested for a more thorough, proportionate and adequate investigation of whether the estrogenic mode of action of Benzophenone might lead to adverse effects in rodents.

Concerning the environment, following up on the concern for estrogenic potential is not straightforward either. Based on the considerations about the environmental fate properties of Benzophenone and its environmental transformation products (c.f. section 7.7), the evaluating MS finds it very uncertain, if further efforts to clarify the estrogenic potential of the metabolites/degradation products of Benzophenone in the context of substance evaluation under REACH would lead to improved risk management of the substance. Further testing would include several steps, possibly first targeting the environmental degradaton products (concerning their chemical identity, their quantitative formation and their further biodegradation rates), followed by endocrine targeted testing of any potentially relevant degradation products known to have estrogenic activity. In addition, there would be a high uncertainty as to whether this might lead to a regulatory relevant outcome in respect to identification of Benzophenone as a SVHC in accordance with REACH art. 57 (f). First of all it is unknown whether relevant degradation products/metabolites such as for example 4-hydroxy Benzophenone would cause adverse effects in fish due to the estrogenic activity, but apart from identification of the substance as an endocrine disruptor in the environment, an SVHC identification also requires an assessment of whether the substance causes equivalent level of concerns as CMR- or vPvB/PBT substances. Based on the available information for Benzophenone regarding the indication for endocrine disruptive effects and environmental fate properties it is considered very uncertain if such a decision would be reached.

In conclusion, the evaluating MS currently does not consider it to be proportionate to request further testing in line with the testing strategy outlined above, which would be required to fully clarify the remaining indications of endocrine properties in regard to wildlife.

Therefore, for the time being, the evaluating MS does not consider this substance to be of priority for further action under substance evaluation.

7.10.1.2. Thyroid disrupting activity

A recent *in vitro* study has shown that Benzophenone decreases the activity of the enzyme thyroid peroxidase (TPO), which is essential in thyroid hormone synthesis. Benzophenone is therefore potentially a thyroid hormone disrupting substance (Song *et al*., 2012).

Several *in vivo* studies have been performed, where some endpoints relevant for thyroid disruption have been assessed (cf. Table 18 below).

Table 18 Overview of studies with potential information regarding thyroid disrupting activity in vitro or in vivo

Description of study	Investigation of effects on Thyroid?	Indication of effects on Thyroid?
TPO inhibition *in vitro* (Song *et al*., 2012)	Yes	Yes
NTP developmental toxicity study, SD rats (NTP, 2002)	No	

NTP developmental toxicity study, NZW rabbits (NTP, 2004a)	No	
Two-generation reproductive toxicity study, dietary exposure, SD rats (Hoshino *et al*., 2005). OECD 416.	Yes Thyroid weight in all dose groups and histology in the highest dose group	No results on the thyroid were reported in the publication, indicating that probably no adverse effects on thyroid gland weight or histopathology were observed.
Carcinogenicity study in Fisher 344 rats (NTP 2006). Dietary exposure for 105 weeks: 15, 30 and 60 mg/kg bw/day. OECD 451.	Yes Thyroid histopathology in all rats	Lower incidences of C-cell hyperplasia in all treated groups of both sexes. No effects on follicular cell adenomas and no adverse effects on follicular cell histopathology.
Carcinogenicity study in B6C3F1 mice (NTP 2006). OECD 451. Doses in diet for 105 weeks: 40, 80, and 160 mg/kg bw/day.	Yes Thyroid histopathology in all mice	No effects on follicular cell adenomas and no adverse effects on follicular cell histopathology.
Subchronic repeated dose toxicity, 14 weeks of dosing in diet, in Fisher 344/N rats. OECD 408. (NTP, 2000)	Yes Thyroid histopathology at 10.000 and 20.000 ppm (i.e. 700 mg/kg bw/day and 850-1000 mg/kg bw/day, respectively)	No adverse effects on thyroid histopathology were noted in male or female mice
Subchronic repeated dose toxicity, OECD 408. 14 weeks of dosing in diet in B6C3F1 mice (NTP, 2000).	Yes Thyriod histopathology at 10.000 and 20.000 ppm	No adverse effects on thyroid histopathology were noted in male or female mice
Subchronic 90 day (28 day) study in rats, dosed in diet with 20, 100 and 500 mg/kg/day. After 28 days all mid and high dose animals and 50% of the low dose animals were sacrificed (Burdock *et al*., 1991).	Yes A number of organs including thyroid was taken from all animals and preserved in formalin	A significant increase in absolute and relative weight of the left thyroid was observed after 90 days of dosing in males from the low dose group (20 mg/kg/d). The two higher dose groups were terminated after 28 days, and no significant effects on thyroid weights were observed.

7.10.1.2.1. Discussion of thyroid disrupting activity – human health

A recent *in vitro* study has shown that Benzophenone decreases the activity of the enzyme thyroid peroxidase (TPO), which is essential in thyroid hormone synthesis. Benzophenone could therefore potentially act as a thyroid hormone disrupting substance (Song *et al*., 2012).

Unfortunately, none of the performed *in vivo* studies included measurements of thyroid hormone levels. Such data could have efficiently clarified whether the rodent thyroid system is affected by Benzophenone *in vivo,* because altered thyroxine levels are often the most sensitive measure of thyroid axis pertubations.

Some details regarding the performed studies are summarized in the table above and in the following text.

In the study by Burdock *et al*. (1991), some significant effects on absolute and relative thyroid weights were reported in Benzophenone exposed animals. This is in contrast to all other Benzophenone *in vivo* studies, where no adverse effects on thyroid gland weight or hitopathology have been observed. In the study by Burdock *et al*., males from the low dose group (20 mg/kg/day), showed a significant increase in absolute and relative weight of the left thyroid after 90 days of dosing. The effect was not seen on the right thyroid or in the females, and was by the authors not considered treatment related. Since rats treated with 100 and 500 mg/kg bw/day (though terminated after only 28 days), showed no significant effects on thyroid weights (Burdock *et al*., 1991), and since exposure to Benzophenone for 2 two years (at dose levels up to 60 mg/kg) did not lead to any observable adverse effects on the thyroid axis, the evaluating MS agrees with the conclusion that the observed effects at 20 mg/kg were probably not treatment related.

Hence, since no adverse effects on thyroid gland weight or histopathology (including no adverse effects on thyroid tumor formation) have been seen in any of the performed subchronic and chronic repeated dose toxicity studies, the overall weight of evidence analysis indicates that Benzophenone is not a thyroid disrupting compound in rodents *in vivo*. This conclusion is based on the fact that even if Benzophenone-induced TPO inhibition does occur in the *in vivo* situation, the consequent decreases in thyroxine production does not seem to be large enough to elicit any observable adverse changes in the rodent thyroid hormone system.

In conclusion, the overall weight of evidence analysis indicates that Benzophenone is not a thyroid disrupting compound in rodents *in vivo*. The evaluating MS therefore does not consider this substance to be of priority for further action under substance evaluation.

7.10.1.2.2. Discussion about thyroid disrupting activity - Environment

The thyroid system is conserved across taxonomic classes, and effects on the thyroid system are relevant to both humans and to wildlife. The observation of inhibition of TPO activity *in vitro* therefore also gives rise to a concern for effects on the thyroid in non-mammalian vertebrate species relevant for the environment (e.g. amphibians).

Even though the overall weight of evidence analysis indicates that Benzophenone is not a thyroid disrupting compound in rodents *in vivo*, there is still a residual concern for thyroid disrupting effect of Benzophenone in non-mammalian vertebrate species (e.g. amphibians) due to the differences in uptake route, possible differences in metabolic transformation between species and possible differences in sensitivity to the thyroid activity of this substance.

During the substance evaluation process, request of the following studies have been considered in order to clarify the concern regarding thyroid disruption in non-mammalian vertebrate species relevant for the environment:
- the Amphibian Metamorphosis Assay (OECD test guideline 231)
and
- the Larval Amphibian Growth and Development Assay (OECD test guideline 241)

However, the available literature indicate that Benzophenone is readily biodegradable as to its fate in surface water. Available information indicates that Benzophenone may be transformed by photolysis and microbes in aquatic systems (Hayashi *et al*., 2006; Chen *et al*., 2015), and several Benzophenone metabolites exist in the environment. When an aqueous solution of Benzophenone is irradiated with UV or sunlight, the two metabolites 3-hydroxy-Benzophenone

(3-OH-BP) and 4-hydroxy-Benzophenone (4-OH-BP) are formed (Hayashi *et al*., 2006). In contrast to the parent substance, Benzophenone, the photolytic transformation product 4-OH-BP (investigation of the other photolytic transformation product 3-OH-BP was not reported) has no effect on the TPO activity *in vitro* (Song *et al*., 2012). Furthermore, the substance is not expected to bioaccumulate in living organisms to any significant extent.

The available information on fate of Benzophenone thus indicate that the substance will be decomposed in the environment in such an extent that the exposure period is expected to be negligible.

All together, there is some residual concern that Benzophenone could induce thyroid toxicity in aquatic wildlife such as amphibians. However the degree of concern is low due to the lack of effects on the thyroid system in rodents *in vivo* as well as its fast transformation of Benzophenone and its low bioaccumulation potential.

7.10.1.2.3. Conclusion – Thyroid disrupting activity

In conclusion, for thyroid disruptive potential, the initial concern was raised by inhibition of TPO activity *in vitro*.

With regards to assessment of the concern for human health, the most sensitive parameter for thyroid disruption (i.e. thyroid hormone levels) was not measured in any of the available *in vivo* rodent studies, leaving some residual concern for thyroid toxicity. However, a large number of long term studies in rodents are available, and thyroid histology and pathology were not affected in any of the studies, including in 2-years carcinogenicity studies in mice and rats. Thus, the overall weight of evidence analysis indicates that Benzophenone is not likely to be a thyroid disrupting substance in rodents *in vivo*.

There is, however, some residual concern that Benzophenone could induce thyroid toxicity in aquatic wildlife such as amphibians. However, the degree of concern is low due to the fast transformation of Benzophenone and its low bioaccumulation potential (see section on environmental fate, 7.7). Therefore, in this specific case, the evaluating MS currently considers it to be too uncertain whether further testing would lead to improved risk management.

Therefore for the time being the evaluating MS does not consider this substance to be of priority for further action under substance evaluation.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

7.12.1. Human health

Examination by the evaluating MS on the exposure scenarios of Benzophenone have resulted in a request to the Registrant(s) to update the exposure evaluations and to update the Chemical Safety Report (CSR) with further details in order to clarify the concern of exposure risks from wide dispersive use. Based on this request the Registrant(s) have provided further details on uses, potential exposures of consumers, industrial and professional workers, and accordingly an updated Chemical Safety Report (CSR).

7.12.1.1. Worker

The evaluating MS requested further information from the Registrant(s) in order to be able to conclude that workers are not at risk due to exposure to Benzophenone. The Registrant(s) have provided further details on exposures: reiterated exposure scenarios, additional exposure scenarios for industrial as well as professional workers and accordingly an updated Chemical Safety Report (CSR).

The evaluating MS has evaluated these additional data and found that the uses described in the updated CSR can be considered as sufficiently specified. These additional data reflects that Benzophenone is used for several industrial productions and professional uses. As a consequence, it seems likely that there is a potential for Benzophenone exposure of industrial as well as professional workers.

Furthermore, as requested by the evaluating MS the Registrant(s) have provided further specifications on the use of personal protective equipment (PPE) like e.g. gloves and respiratory protection, which is recommended in the technical dossier. The evaluating MS has concluded that the additional data in the updated CSR regarding PPEs (e.g. type of material, thickness and breakthrough times of the gloves) were sufficiently specified in the updated CSR.

Based on the provided new information OC and RMMs at workplace seems to be sufficient and workers seems not to be at immediate risk.

7.12.1.2. Consumer

Examination by the evaluating MS on the exposure scenarios of Benzophenone has resulted in a request to the Registrant(s) for further details on possible consumer exposure.

As a consequence, the Registrant(s) have provided an update of the Chemical Safety Report (CSR) containing further information and details in order to clarify the concern for consumer exposure due to wide dispersive use of Benzophenone.

Consumer scenarios are developed for consumer use of ink and toners (change of cartridges, printer operation 2h/day) and service life of articles and materials – use of painted or coated articles and consumer use of wet wipes.

Some end-uses of Benzophenone and the corresponding products are already regulated under other directives not included in REACH. This includes cosmetic products (fragrances, cosmetics), personal care and pharmaceuticals. No human exposure scenarios has been developed for these scenarios as they are not required under REACH and regulated under the corresponding legislations for pharmaceuticals or cosmetic products, respectively. However, the corresponding tonnages have been included into the environmental exposure assessment. Furthermore, the Registrant(s) state that no Benzophenone is used in coatings with food contact, i.e. no migration into food or drink possible.

According to REACH article 14.2 for products with Benzophenone concentrations below 1% no exposure estimation is demanded. Therefore, the Registrant(s) have only been taken scenarios with a minimum Benzophenone concentration in the product of 1 % into account.

The evaluating MS has evaluated the additional data submitted by the Registrant(s) on the uses of Benzophenone and found that the exposures described in the CSR can be considered as sufficiently specified, as they reflect that Benzophenone is used in several consumer products and it seems likely that there is a potential for Benzophenone exposure of consumers.

Even though consumer exposure is possible and that there is a wide dispersive use of Benzophenone, it can be concluded that the developed scenarios:

- consumer use of ink and toners (change of cartridges, printer operation 2h/day),

- service life of articles and materials – use of painted or coated articles, and
- consumer use of wet wipes

do not demonstrate that humans (consumers) are at risk according to the REACH regulation.

7.12.2. Combined exposure assessment

The evaluating MS has concluded that combined scenarios for all exposure routes are relevant for Benzophenone and has therefore requested the Registrant(s) to develop combined exposure scenarios or as a minimum to submit a justification for the statement that combined scenarios for all exposure routes are: "Not relevant" (i.e. in that case to submit the relevant documentation for the adequacy of this statement). Based on this request the scenarios were provided by the Registrant(s) in an updated CSR and were subsequently assessed to be sufficiently described.

Even though there is a potential for worker and consumer exposure to Benzophenone, the evaluating MS has concluded that the combined exposure assessment does not demonstrate that humans (workers and consumers) are at risk according to the REACH regulation. This conclusion covers the identified uses of Benzophenone.

7.13. Risk characterisation

The evaluating MS has initially identified wide dispersive use and high risk characterization ratios (RCRs) related to worker exposure to Benzophenone via the inhalation and dermal routes. In many of the scenarios/contributing scenarios RCRs have been estimated to be 1 and in the close vicinity to 1 (>0.91 - 1). High RCRs indicate a need for further clarification, and for that reason the evaluating MS requested a refinement of the exposure scenarios by the use of e.g. higher Tier tools, and further information on RMMs (Risk Management Measures).

Based on new and detailed information from the Registrant on OCs, RMMs and a refinement of the exposure scenarios in the updated CSR the evaluating MS concluded that the described exposure to Benzophenone does not demonstrate that humans (workers and consumers) are at risk according to the REACH regulation.

7.14. References

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7.15. Abbreviations

3-OH BP: 3-hydroxybenzophenone

4-OH BP: 4-hydroxybenzophenone

ALP: alkaline phosphatase

AMA: Amphibian Metamorphosis Assay

ARE-luciferase: antioxidant response element - luciferase

BCF: bio-concentration factor

BMDL: The lowest Benchmark dose

BP: Benzophenone

Bw: body weight

CEF: the Scientific Opinion of the Panel on food contact materials, enzymes, flavourings and processing aids

CMR: carcinogenic, mutagenic and reproductive toxic substances

CoRAP: Community Rolling Action Plan

CSR: Chemical Safety Report

DMSO: Dimethylsulfoxide

DNEL: derived No Effect level

EAWAG: Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz (in English: Federal Institute of Aquatic Science and Technology (in Switzerland))

ECHA: The European Chemicals Agency

EC_{50}: effect concentration for 50 %

ED: endocrine disruptor

EFSA: The European Food Safety Authority

EOGRTS: Extended One-Generation Toxicity Study

ER (binding/activation): Estrogen receptor (binding/activation)

Evaluating MS: evaluating Member state

F0: parent generation

F1: first generation

F2: second generation

FSH: Follicle-stimulating hormone

FSDT: Fish Sexual Development Test

GFP: green fluorescent protein

GLP: Good Laboratory Practice

HSDB: Hazardous Substances Data Bank

KEGG Pathway Predictor: Kyoto Encyclopedia of Genes and Genomes PATHWAY database that consists of graphical diagrams of biochemical pathways including most of the known metabolic pathways and some of the known regulatory pathways.

LAGDA: Larval Amphibian Growth and Development Assay

LH: Luteinizing hormone

LMS: lysosomal membrane stability

LOAEL: lowest observed adverse effect level

LC_{50}: lethal concentration for 50 %

logKoa: Logarithm to octanol-air coefficient

logKow: Logarithm to octanol-water coefficient

MCF-7: human breast adenocarcinoma cell line

MNCL: mononuclear cell leukemia

NOAEL: No Observed Adverse Effect Level

NOEC: No Observed Adverse Effect Concentration

NZW rabbits: New Zealand White Rabbits

OC: operational conditions

OECD: Organisation for Economic Co-operation and Development

OVX SD rats: ovariectomized Sprague Dawley rat

PPE: Personal protective equipment

vPvB/ PBT: very persistent and very bio-accumulative/ persistent, bio-accumulative and toxic

QSAR: Quantitative Structure-Activity Relationship

RCR: risk characterization ratio

RGCs: radial glial cells

RMM: Risk Management Measure

SD rat: Sprague Dawley rat

STP sludge: sewage treatment plant sludge

SVHC: substances of very high concern

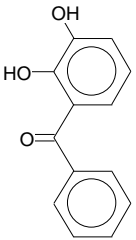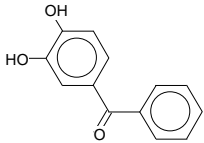T3: triiodothyronine

T4: thyroxine
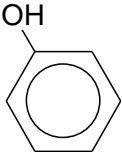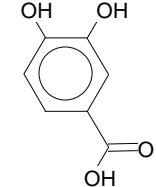
TDI: Tolerable daily intake

TPO: thyroid peroxidase

TSH: Thyroid-stimulating hormone

7.16. Appendix

Table 1: Aromatic structures indicated by OECD Toolbox microbial degradation simulator as metabolites of biotic degradation of Benzophenone and the predictions of estrogen disruptive properties of these. "POS" means that the prediction is positive for this endpoint, "NEG" means that the prediction is negative for this endpoint, INC" means that the prediction is inconclusive, "IN" means that the prediction is within applicability domaine, "OUT" means that prediction is out of applicability domaine, "EXP" means that this is based on experimental data and not a prediction.

Identifiers Name CAS no. Smilies	OECD Toolbox (Estrogen Receptor α Binding)	DK QSAR DB Estrogen Receptor α Binding, Full training set (Human *in vitro*)	DK QSAR DB Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)	DK QSAR DB Estrogen Receptor α Activation (Human *in vitro*)	DK QSAR DB Androgen Receptor Antagonism (Human *in vitro*)	Structure
(2,3-dihydroxy-phenyl)-phenyl-methanone CAS # NA Oc1cccc(C(=O)c2ccccc2)c1O	Strong binder	NEG_IN	INC_OUT	NEG_IN	POS_IN	
(3,4-dihydroxy-phenyl)-phenyl-methanone CAS # 10425-11-3 Oc1ccc(C(=O)c2ccccc2)cc1O	Strong binder	INC_OUT	POS_IN	POS_IN	POS_IN	

Benzoic acid CAS # 65-85-0 OC(=O)c1ccccc1	Non-binder	NEG_IN	NEG_IN	NEG_IN	NEG (EXP)	
Phenols, petroleum CAS # 64743-03-9 Oc1ccccc1	Weak binder, OH group	No predictions available for UVCBs For pure phenol: NEG (EXP)	No predictions available for UVCBs For pure phenol: NEG (EXP)	No predictions available for UVCBs For pure phenol: NEG_IN	No predictions available for UVCBs For pure phenol: NEG_IN	
3,4-dihydroxy-benzoic acid CAS # 99-50-3 Oc1ccc(C(O)=O)cc1O	Weak binder, OH group	INC_OUT	INC_OUT	NEG_OUT	NEG_IN	
Catechol CAS # 120-80-9 Oc1ccccc1O	Weak binder, OH group	NEG (EXP)	NEG (EXP)	NEG_IN	NEG_IN	
Bis(2,3-dihydroxyphenyl)-methanone CAS # NA Oc1cccc(C(=O)c2cccc(O)c2O)c1O	Very strong binder, OH group	INC_OUT	POS_OUT	NEG_IN	INC_OUT	

(2,3-dihydroxy-phenyl) -(3,4-dihydroxy-phenyl)- methanone CAS # 37728-15-7 Oc1cccc(C(=O)c2ccc(O)c(O)c2)c1O	Very strong binder, OH group	POS_OUT	POS_IN	INC_OUT	POS_IN	