**Committee for Risk Assessment**

**RAC**

Annex 1

**Background document**

to the Opinion proposing harmonised classification and labelling at EU level of

# 4-phenylbenzophenone

**EC Number: 218-345-2**
**CAS Number: 2128-93-0**

CLH-O-0000007379-62-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It is based on the official CLH report submitted to consultation and additional information (if applicable).

**Adopted**

**30 November 2023**

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# CLH report

# Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),**
**Annex VI, Part 2**

# Chemical name:
# 4-Phenylbenzophenone

**EC Number:** **218-345-2**

**CAS Number:** **2128-93-0**

**Index Number:** **-**

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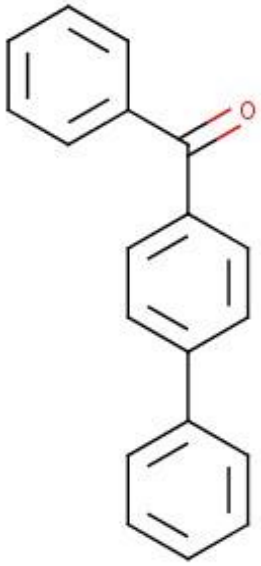
**Version number: 1.0** **Date: February 2023**

# CONTENTS

# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

**Table 1: Substance identity and information related to molecular and structural formula of the substance**

| | |
|---|---|
| **Name(s) in the IUPAC nomenclature or other international chemical name(s)** | 4-phenylbenzophenone |
| **Other names (usual name, trade name, abbreviation)** | 4-benzoylbiphenyl; biphenyl-4-yl(phenyl)methanone |
| **ISO common name (if available and appropriate)** | n.a. |
| **EC number (if available and appropriate)** | 218-345-2 |
| **EC name (if available and appropriate)** | 4-phenylbenzophenone |
| **CAS number (if available)** | 2128-93-0 |
| **Other identity code (if available)** | - |
| **Molecular formula** | $C_{19}H_{14}O$ |
| **Structural formula** |  |
| **SMILES notation (if available)** | O=C(C1=CC=CC=C1)C1=CC=C(C=C1)C1=CC=CC=C1 |
| **Molecular weight or molecular weight range** | 258.32 g/mol |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | > 99 |

## 1.2 Composition of the substance

**Table 2: Constituents (non-confidential information)**

| Constituent (Name and numerical identifier) | Concentration range (% w/w minimum and maximum in multi-constituent substances) | Current CLH in Annex VI Table 3 (CLP) | Current self-classification and labelling (CLP) |
|---|---|---|---|
| 4-phenylbenzophenone (CAS No. 2128-93-0, EC No. 218-345-2) | > 99 | No harmonized classification | Repr. 1B - H360<br>Aquatic Acute 1 - H400<br>Aquatic Chronic 1 - H410<br>Skin Sens. 1B - H317<br>Skin Sens. 1 - H317 |

**Table 3: Test substances in toxicological studies**

| Identification of test substance | Purity | Impurities and additives (identity, %, classification if available) | Other information | The studies in which the test substance is used |
|---|---|---|---|---|
| **Omnirad 4 PBZ, Batch 2128-93-0** | 4-phenylbenzophenone 99.77 % | | Vehicle used: Propylene glycol | Combined 28-day repeated dose toxicity study |
| **Omnirad 4 PBZ, Batch 20161118** | 4-phenylbenzophenone 99.74 % | | Vehicle used: Acetonitrile | DPRA test |
| **Omnirad 4 PBZ, Batch 20161118** | 4-phenylbenzophenone 99.74 % | | Vehicle used: Dimethyl sulfoxide | KeratinoSens TM assay |
| **Omnirad 4 PBZ, Batch 20161118** | 4-phenylbenzophenone 99.74 % | | Vehicle used: N,N-dimethylformamide | LLNA assay |
| **Omnirad 4 PBZ, Batch 20161118** | 4-phenylbenzophenone 99.74 % | | Vehicle used: Dimethyl sulfoxide | AMES test (test facility study number 518662) |
| **Omnirad 4 PBZ, Batch 20161118** | 4-phenylbenzophenone 99.74 % | | Vehicle used: Dimethyl sulfoxide | AMES test (test facility study number 20142208) |

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

**Table 4: 4-phenylbenzophenone**

| | Index No | Chemical name | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors and ATEs | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitter's proposal | TBD | 4-phenylbenzophenone | 218-345-2 | 2128-93-0 | Repr. 1B<br>Skin Sens. 1B<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H360FD<br>H317<br>H400<br>H410 | GHS08<br>GHS07<br>GHS09<br>Dgr | H360FD<br>H317<br>H410 | | M = 10<br>M = 1 | |
| Resulting Annex VI entry if agreed by RAC and COM | | | | | | | | | | | |

**Table 5: Reason for not proposing harmonised classification and status under consultation**

| Hazard class | Reason for no classification | Within the scope of consultation |
|---|---|---|
| **Explosives** | Hazard class not assessed in this dossier | No |
| **Flammable gases (including chemically unstable gases)** | | |
| **Oxidising gases** | | |
| **Gases under pressure** | | |
| **Flammable liquids** | | |
| **Flammable solids** | | |
| **Self-reactive substances** | | |
| **Pyrophoric liquids** | | |
| **Pyrophoric solids** | | |
| **Self-heating substances** | | |
| **Substances which in contact with water emit flammable gases** | | |
| **Oxidising liquids** | | |
| **Oxidising solids** | | |
| **Organic peroxides** | | |
| **Corrosive to metals** | | |
| **Acute toxicity via oral route** | | |
| **Acute toxicity via dermal route** | | |
| **Acute toxicity via inhalation route** | | |
| **Skin corrosion/irritation** | | |
| **Serious eye damage/eye irritation** | | |
| **Respiratory sensitisation** | | |
| **Skin sensitisation** | **Skin Sens. 1B** | **Yes** |
| **Germ cell mutagenicity** | Hazard class not assessed in this dossier | No |
| **Carcinogenicity** | | |
| **Reproductive toxicity** | **Repr. 1B** | **Yes** |
| **Specific target organ toxicity-single exposure** | Hazard class not assessed in this dossier | No |
| **Specific target organ toxicity-repeated exposure** | | |
| **Aspiration hazard** | | |
| **Hazardous to the aquatic environment** | **Aquatic Acute 1**<br>**Aquatic Chronic 1** | **Yes** |
| **Hazardous to the ozone layer** | Hazard class not assessed in this dossier | No |

## 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification for this substance.

## 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

***Concerning classification for reproductive toxicity:***

There is no requirement for justification that action is needed at Community level.

***Concerning classification for skin sensitisation:***

Justification that action is needed at Community level is required.

The following reasoning is provided by the dossier submitter (DS): As of December 2021, there are differences in self-classification reported by the notifiers; besides, the DS disagrees with part of the available self-classifications (ECHA, 2021):

Skin Sens. 1: 1 out of 2365

Skin Sens. 1B: 95 out of 2365

No classification: 2269 out of 2365

***Concerning classification for hazardous to the aquatic environment:***

**Justification that action is needed at Community level is required.**

**Reason for a need for action at Community level:**

*Disagreement by DS with current self-classification*
*Differences in self-classification*

Notified classification and labelling for hazardous to aquatic environment are inconsistent and contradictory as seen below (as of October 2022):

Aquatic Acute 1 + Aquatic Chronic 1: 96 of 2365 (different M-factors)

No classification: 2269 of 2365

## 5 IDENTIFIED USES

The substance is used as a photo-initiator in multiple applications, such as graphic arts, wood coatings, plastic coatings, metal coatings, electronics, or adhesives to induce polymerisation of unsaturated oligomers, such as acrylates (IGM Resins B.V., 2021). Similarly, PubChem database reports use in/as paints and coatings, which falls under the category "consumer uses", according to the US EPA (PubChem, 2021).

Based on the ECHA substance dissemination web-page (Substance Infocard), there are no uses by consumers reported by the notifiers in the EU (ECHA, 2021):

*"This substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 10 to < 100 tonnes per annum. This substance is used by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.*

*Consumer Uses: ECHA has no public registered data indicating whether or in which chemical products the substance might be used. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment."*

Nevertheless, 4-phenylbenzophenone (CAS 2128-93-0) has been listed in section B of the "Ordinance of the Swiss Federal Department of Home Affairs on materials and articles intended to come into contact with foodstuffs" (SR 817.023.21) (FSVO, 2020). Therefore, consumer exposure may arise from contact to the food packaging which contains UV-curing inks with 4-phenylbenzophenone serving as a photo-initiator (Bentayeb et al., 2013). An oral exposure cannot be excluded.

## 6 DATA SOURCES

The primary source of data used in this report are the full study reports provided by the registrant (REACH registration) in November 2021 upon request of the DS made in October 2021. The submission included all

requested studies, specifically: a combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test, an *in vitro* skin corrosion test, an *in vitro* skin irritation test, a bovine corneal opacity and permeability assay, a DPRA (direct peptide reactivity assay), a KeratinoSens™ assay, an LLNA, a chromosome aberration study, an Ames test (test facility study number 518662), a second Ames test (test facility study number 20142208), and a QSAR report informing on the DEREK-derived prediction of the skin sensitisation properties of 4-phenylbenzophenone.

Other consulted sources included the substance-specific ECHA dissemination web-page, the registration dossier (full, joint submission) and CSR. Additionally, the DS conducted two literature reviews, one in January, 2019 and another one in August, 2020, yielding 36 relevant publications. The literature search covered seven literature databases: Scopus, Science Direct, Web of Science, PubMed, Wiley Online Library, Embase and TOXLINE (incl. PubMed). The search string included the IUPAC as well as other names and identifiers of the substance: methanone, (1,1'-biphenyl)-4-ylphenyl-/4-phenylbenzophenone/benzophenone, 4-phenyl-/4-benzoylbiphenyl (CAS-Nr. 2128-93-0 / EC-Nr. 218-345-2).

## 7 PHYSICOCHEMICAL PROPERTIES

**Table 6: Summary of physicochemical properties**

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20 °C and 101.3 kPa** | White to off-white solid, particulate/powder | REACH registration data | - |
| **Melting/freezing point** | 101.5 °C at 1015 hPa | REACH registration data | Measured: OECD 102, EC A.1, OPPTS 830.7200 (DSC) |
| **Boiling point** | 421.8 °C at 1015 hPa | REACH registration data | Measured: OECD 103, EC A.2., OPPTS 830.7220 (DSC) |
| **Relative density** | 1.26 g/cm³ at 20 °C | REACH registration data | Measured: OECD 109, EC A.3., OPPTS 830.7300 (gas comparison stereopycnometer) |
| **Vapour pressure** | $\leq 1.9*10^{-5}$ Pa at 20 °C<br>$\leq 4.3*10^{-5}$ Pa at 25 °C | REACH registration data | Measured: OECD 104, EC A.4., OPPTS 830.7950 (effusion: isothermal thermogravimetry) |
| **Surface tension** | Waived: Water solubility (< 1 mg/L at 20 °C) | REACH registration data | - |
| **Water solubility** | 0.0736 mg/L at 20 °C, pH 6.5 | REACH registration data | Measured: EC A.6, OECD 105, OPPTS 830.7840 (HPLC) |
| **Partition coefficient n-octanol/water** | log $P_{ow}$ = 4.7 at 35 °C, pH 7 | REACH registration data | Measured: OECD 117, EC A.8, EC A.24, OPPTS 830.7570 (HPLC) |
| **Flash point** | Waived: Not relevant (no low melting point solid) | REACH registration data | - |
| **Granulometry** | $d_{10}$ = 127.167 µm±4.524<br>$d_{50}$ = 284.989 µm±3.36<br>$d_{90}$ = 494.367 µm±6.314 | REACH registration data | ISO 13320 and CIPAC MT 187 (Laser scattering/diffraction) |

The information in this table marked with "REACH registration" data is taken from the REACH registration dossier and ECHA's public registration information as accessed on 27-10-2021.

# 8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

## 9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No available studies covering this endpoint were submitted by the registrant(s) or found in the literature.

Based on its physico-chemical characteristics, the substance is poorly water-soluble (0.0736 mg/L at 20 °C, pH 6.5) and lipophilic (log Pow = 4.7 at 35 °C, pH 7). The volatility of the substance is low, with vapour pressure values below or equal to 1.9 x $10^{-5}$ Pa at 20 °C and below or equal 4.3 x $10^{-5}$ Pa at 25 °C. Based on the molecular weight of 258.32 g/mol, the low water solubility and the log Pow of 4.7, limited oral and dermal absorption would be expected. However, the Danish (Q)SAR Database was as well applied to predict oral and skin absorption by using the EC number of 4-phenylbenzopenone as a unique identifier to search for available predictions saved in the database (EPA-DK, 2021). Based on the Lipinski's rule of five, high oral bioavailability was predicted, with estimated absorption efficiency from the gastrointestinal tract of 100 % and 90 % for doses of 1 and 100 mg of 4-phenylbenzophenone respectively. Low skin absorption was predicted by the EPI DERMWIN model, estimated at 0.000506 mg/$cm^2$/event. A high log brain/blood partition coefficient of 0.8753 was calculated for 4-phenylbenzophenone. Based on the low vapour pressure, inhalation uptake is expected to be low.

In the absence of experimental data, a publicly available physiologically based pharmacokinetic (PBPK) model (Pearce et al., 2017) was run to generate a putative prediction of distribution of 4-phenylbenzophenone. The exposure conditions of the combined 28-day toxicity study were selected as input, namely 100 mg/kg bw/d of 4-phenylbenzophenone given orally for 28 days. The model suggests distribution of the parent substance in different compartments of the rat body, with achieved $C_{max}$ values aligning in the following order: $C_{gut} > C_{liver} > C_{kidney} > C_{lung} > C_{rest} > C_{plasma} > C_{art} > C_{venous}$. The predicted median $C_{max}$ values ranged from 2.142 M in liver to 85 µM in plasma.

# 10 EVALUATION OF HEALTH HAZARDS

## 10.1 Acute toxicity

Not assessed in this report

## 10.2 Skin corrosion/irritation

Not assessed in this report

## 10.3 Serious eye damage/eye irritation

Not assessed in this report

## 10.4 Respiratory sensitisation

Not assessed in this report

## 10.5 Skin sensitisation

**Table 7: Summary table of animal studies on skin sensitisation**

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels duration of exposure | Results | Reference |
|---|---|---|---|---|---|
| Local lymph node assay (LLNA)<br><br>OECD TG 429<br><br>GLP | Female CBA:J mice, (5/group) | 4-phenylbenzophen-one, identified as Omnirad 4-PBZ<br>Batch: 20161118<br>Purity: 99.74 % | 5, 10 or 25 % w/w in N,N-dimethyl-formamide on 3 consecutive days by open application on the ears; 3 days after the last exposure all mice were injected with $^{3}$H-methylthymidine<br><br>Positive control was not included for scientific and animal welfare reasons. | **Positive**<br><br>Mean SI values (at test concentrations):<br><br>1.3 (5 %)<br><br>2.3 (10 %)<br><br>2.9 (25 %) | (Charles River, 2018a)<br><br>Reliability: 1 |

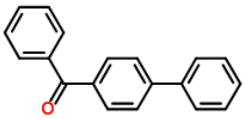
**SI: stimulation index**

**Table 8: Summary table of human data on skin sensitisation**

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No data on the effects of 4-phenylbenzophenone on human skin sensitisation were available | | | | |

**Table 9: Summary table of other studies relevant for skin sensitisation**

| Type of study/data | Test substance, batch, purity | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| *In vitro* skin sensitisation: ARE-Nrf2[1] luciferase test<br><br>KeratinoSens™ assay<br><br>OECD TG 442D<br><br>GLP* | 4-phenylbenzophenone, identified as Omnirad 4-PBZ<br>Batch: 20161118<br>Purity: 99.74 % | Omnirad 4-PBZ in dimethyl sulfoxide;<br><br>2 concentration ranges:<br><br>1st Exp.: 0.06 – 125 µM<br><br>2nd Exp.: 0.0005 – 1 µM<br><br>KeratinoSens™ cells with ARE-Nrf2 reporter were incubated with respective concentrations of | KeratinoSens™ assay: **positive**<br><br>Induction of ARE:<br><br>**1st experiment** (0.06 – 125 µM)**:**<br><br>$EC_{1.5}$ < 0.06 µM and $I_{max}$ = 18.84, cytotoxicity observed ($IC_{30}$ = 37 µM and $IC_{50}$ = 52 µM)<br><br>**2nd experiment** (0.0005 – 1 µM)**:**<br><br>$EC_{1.5}$ = 0.64 µM and $I_{max}$ = 1.7 at cell viability >70 %<br><br>Positive control: acceptable | (Charles River, 2017b)<br><br>Reliability: 1 |

| Type of study/data | Test substance, batch, purity | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | Omnirad 4-PBZ for 48 hours<br>Positive control: Ethylene dimethacrylate glycol<br>Read-out for ARE-pathway activation: Luciferase-based luminescence<br>Cell viability: MTT assay, spectrophotometry, absorption at 570 nm | 1st experiment: $EC_{1.5}$ = 24 µM and $I_{max}$ = 3.7<br>2nd experiment: $EC_{1.5}$ = 39 µM and $I_{max}$ = 2.53<br>Variation: acceptable<br>(CV < 20 % in 1st and 2nd experiments in DMSO neg. ctrl.) | |
| *In chemico* skin sensitisation: direct peptide reactivity assay (DPRA)<br>OECD TG 442C<br>GLP | 4-phenylbenzophenone, identified as Omnirad 4-PBZ<br>Batch: 20161118<br>Purity: 99.74 % | 100 mM Omnirad 4-PBZ diluted in acetonitrile; incubated for 24 hours at 25 ºC with synthetic peptides SPCC[2] or SPCL[3]. After incubation, the remaining concentrations of peptides were determined using HPLC-PDA[4]. | DRPA prediction: **negative**<br>Reactivity class: no or minimal reactivity class<br>Mean of SPCC and SPCL depletion: 1.5 % at 100 mM<br>SPCC depletion: 0.6±1.1 % (mean ±SD)<br>SPCL depletion: 2.5±2.7 % (mean ±SD)<br>**Limitation:** test item precipitation, limiting the amount of test item in the solution required for interaction with the SPCL peptide | (Charles River, 2017c)<br>Reliability: 1 (GLP certificate was provided in the study report. However, due to the main limitation of the experiment (precipitation), the negative results should be interpreted with caution). |
| *In silico* skin sensitisation:<br>QSAR<br>Derek prediction on skin sensitisation<br>GLP not applicable | 4-phenylbenzophenone<br>CAS Number: 2128-93-0,<br>Molecular weight: 258.3,<br>Molecular formula: $C_{19}H_{14}O$<br>Structure:<br>  | Derek Nexus ver. 5.0.2, Nexus 2.1.1<br>Knowledge Database: Derek KB 2015 2.0 | QSAR prediction: **negative**<br>No alerts for skin sensitisation | (Charles River, 2017a) |

**[1]ARE: antioxidant/electrophile responsive element**

**[2]SPCC: synthetic peptides containing cysteine**

*[3]SPCL: synthetic peptides containing lysine*
*[4]HPLC-PDA: high-performance liquid chromatography with gradient elution and photodiode array detection*
**In spite of the absence of a GLP certificate in the full study report, the study was conducted in a GLP certified laboratory, thus the reliability of the study was assessed as "reliable without restrictions"*

### 10.5.1 Short summary and overall relevance of the provided information on skin sensitisation

***In vivo* data**

One *in vivo* animal study is available for 4-phenylbenzophenone. In a reliable, GLP-compliant LLNA assay in mouse (strain: CBA:J), a dose-dependent increase in local lymphocyte proliferation was observed (see Figure 1), demonstrating that 4-phenylbenzophenone has skin sensitising properties (Charles River, 2018a). At the highest concentration tested (25 % w/w), SI values of 2.9 (mean) and 3.8 (median) were reported, suggesting that 4-phenylbenzophenone is a skin sensitiser of moderate potency.

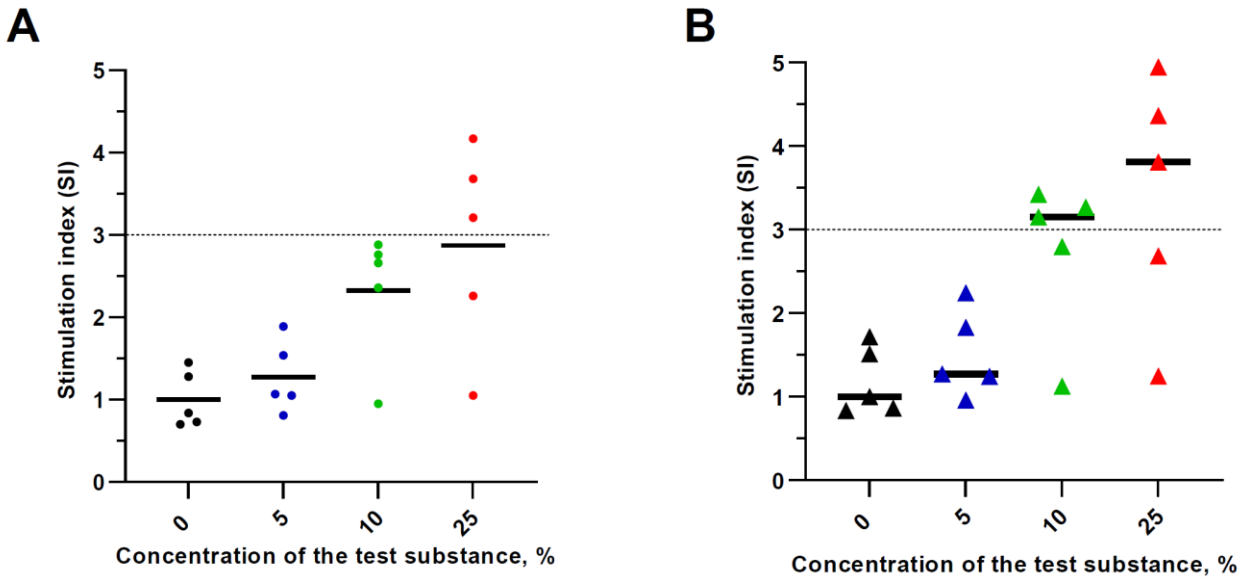



Figure 1: Effect of 4-phenylbenzophenone on local lymphocyte proliferation in LLNA study.
A) Data distribution is presented as individual stimulation indices (SI) calculated from individual disintegrations per minute (DPM) divided by the group mean DPM of the vehicle control (dots); the group mean SI are represented as solid lines.
B) Same as panel A), but instead individual SI were calculated by dividing DPM for each animal by the group median DPM of the vehicle control (triangles); the median SI of the treatment groups are represented by thick lines. SI of 3 is shown for reference in both panels (dotted line).

**The study was performed according to OECD TG 429, with a deviation of maximum tested concentrations (25 % instead of 50 %), but this deviation does not undermine the overall study quality. The provided justification that testing at higher concentrations had not been conducted due to the poor solubility of 4-phenylbenzophenone in the vehicle N,N-dimethylformamide, resulting in low homogeneity of ≥ 30 % solution of 4-phenylbenzophenone, is considered acceptable.**

Based on the results of the test, where mean SI = 2.9 at the concentration level of 25 %, it is considered to be a borderline case. Nevertheless, the criteria of SI ≥ 3 are considered to be fulfilled, because the variability at high dose depends mainly on one very low DPM value, which has a large influence on the arithmetic mean (see Figure 1 A). Calculation of the median SI instead of the arithmetic mean results in an SI of 3.8 at high dose (25 %) and is clearly above the cut-off value of 3 (see Figure 1 B). Already the median SI of 3.2 at mid dose (10 %) is above 3.

Consequently, effective concentration inducing a stimulation index of 3 (rounded) in the LLNA test was determined at the concentration level of 25 % (EC3 > 2 %).

***In silico/in chemico/in vitro* data**

One *in silico* prediction was conducted using Derek Nexus v. 5.0.2 knowledge-based QSAR system, employing the Derek KB 2015 2.0 knowledge database (Charles River, 2017a). Derek predicted that 4-phenylbenzophenone did not contain any of the 80 alerts for skin sensitisation known to the system. Considering the recommendation of the ECHA guidance on the application of the CLP criteria (ver. 5), more weight should be given to the good quality data on the substance itself vs. extrapolations from similar substances. Therefore, the prediction generated by Derek Nexus v. 5.0.2 is given lower weight in the current weight of evidence assessment, as it is based on the training set containing similar substances, while available *in vivo* and *in vitro* studies (discussed below) were conducted with 4-phenylbenzophenone itself. Additionally, the most recent OECD TG 497 on defined approaches for skin sensitisation requests use of a newer version of Derek software for the prediction of skin sensitising properties, i.e. Derek Nexus v.6.1.0.

One DPRA *in chemico* test was conducted with 4-phenylbenzophenone, which investigated the potential of binding of the substance to cysteine- and lysine-containing synthetic peptides (Charles River, 2017c). In this study, low cysteine and lysine reactivity of 4-phenylbenzophenone was predicted, contradicting the *in vivo* findings. As a potential explanation for this result, the study authors suggested that precipitation of 4-phenylbenzophenone upon addition to the solution with the SPCL peptide may have had an impact on the availability of the substance for reaction with peptides. This corresponds to the OECD TG 442C recommendation to interpret the negative result with due care if a precipitate is observed immediately upon addition of the test chemical solution to the peptide solution (OECD, 2021b).

One *in vitro* assay is available to further support the classification as skin sensitiser of 4-phenylbenzophenone in a weight-of-evidence assessment (Charles River, 2017b). The KeratinoSens$^{TM}$ assay with 4-phenylbenzophenone was positive and demonstrated a 1.5-fold induction at the concentration of 0.64 µM and a 1.7-fold maximal induction of the ARE-response pathway, while no cytotoxicity was observed at this dose level. For comparison, a positive control used in this assay caused 1.5-fold induction at 24 µM and maximal 3.7-fold induction in one of two independent experiments (2$^{nd}$ experiment: $EC_{1.5}$=39 µM and $I_{max}$=2.53). However, the application of the "2 out of 3" defined approach (2o3DA) is restricted due to the lack of further *in vitro* data (dendritic cell activation test, h-CLAT) and the results of the DPRA assay, which should be taken with care (OECD, 2021a).

Overall, the reliable GLP-compliant LLNA study is considered a key study relevant for the classification.

### 10.5.2 Comparison with the CLP criteria

According to the CLP criteria for skin sensitisers, Annex I: 3.4.2.2.1.1 of CLP Regulation 1272/2008:
"*Skin sensitizers shall be classified in Category 1 where data are not sufficient for sub-categorisation.*"

There are sufficient data in the current CLH dossier to allow for sub-categorisation. Therefore, classification in Category 1 is not justified.

In accordance with the criteria given in Table 3.4.2 of the CLP Regulation 1272/2008, substances should be classified to sub-category 1A if: *"Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered"(ECHA, 2017).*

Data were not available to assess frequency of occurrence or severity of skin sensitisation reactions to 4-phenylbenzophenone in humans. In one available animal test in conformity with OECD TG 429, the local lymph node assay, the EC3 value was higher than 2 %, a limit concentration defining the threshold for skin sensitisers of high potency according to Table 3.4.3 of the CLP Regulation 1272/2008. Therefore, classification to sub-category 1A is not applicable.

However, substances should be classified as skin sensitisers of sub-category 1B according to the criteria stated in Table 3.4.2 of the CLP Regulation 1272/2008 if: *"Substances showing a low to moderate frequency*

*of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered".*

As stated above, human data were not available for 4-phenylbenzophenone. In animals, the results of a reliable local lymph node assay demonstrated **moderate potency** of 4-phenylbenzophenone, with the **EC3 value = 25 %** (rounded, w/v), determined at the highest achieved tested concentration. According to Table 3.4.4 of the CLP Regulation 1272/2008, this result warrants **a classification as skin sensitiser (Category 1B) for 4-phenylbenzophenone**.

### 10.5.3 Conclusion on classification and labelling for skin sensitisation

In conclusion, the DS proposes to classify 4-phenylbenzophenone (EC 218-345-2) as Skin Sens. 1B (H317 - May cause an allergic skin reaction). For the classification of mixtures, the generic concentration limit (GCL) of 1 % (w/v) should be applied.

## 10.6 Germ cell mutagenicity

This endpoint was not assessed due to the existing data gap. The DS is aware of the ongoing compliance check for 4-phenylbenzophenone. Currently, based on the two available positive Ames tests, germ cell mutagenicity cannot be excluded (Charles River, 2018c; Charles River, 2018d). However, in the absence of required follow-up studies, neither the hazard can be ruled out nor classification into the correct hazard class can be proposed.

## 10.7 Carcinogenicity

Not assessed in this report

## 10.8 Reproductive toxicity

### 10.8.1 Adverse effects on sexual function and fertility

**Table 10: Summary table of animal studies on adverse effects on sexual function and fertility[1],[2]**

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| Combined 28-day repeated dose toxicity study with the reproduction /developmental toxicity screening test | 4-phenylbenzophenone, identified as Omnirad 4-PBZ<br><br>Purity: 99.74 %<br><br>Dose levels (oral, gavage): 0, 100, 300, 1000 mg/kg bw/day<br><br>Vehicle: Propylene glycol<br><br>Frequency of exposure: Once | **Parental NOAEL** = 300 mg/kg bw/d (based on increased incidences of thyroid follicular cell hypertrophy in males and females and inflammatory cell infiltration in adrenals in females at 1000 mg/kg bw/d)<br><br>**Reproductive NOAEL** = 300 mg/kg bw/d (based on reduced number of implantation sites at 1000 mg/kg bw/day)<br><br>**Reproductive toxicity**: | (Charles River, 2018b)<br><br>**Key study**<br><br>Klimisch Score 1 |

[1] In the current table pre-implantation effects are described

[2] Non-adverse effects are summarised for transparency of reporting. **Adverse effects** are marked in bold to improve understanding of the position of DS.

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| OECD TG 422<br><br>GLP<br><br>Male and female Crl:WI(Han) rats (10/ sex/dose) | daily, 7 days a week<br><br>Duration of exposure:<br>Males were treated for 29 days, including 14 days prior to mating. Females that delivered were treated for 50-56 days, including 14 days prior to mating and 13-15 days after the delivery. Other females were treated for 39-43 days (1000 mg/kg bw/d) or 41-54 days (100 and 300 mg/kg bw/d). | - at 1000 mg/kg bw/d stat. sign. ($p<0.01$, Steel test) **reduced mean number of implantation sites** (13.0, 11.8, 12.0, 9.5** at 0, 100, 300 and 1000 mg/kg bw/day, respectively corresponding to -9.2, -7.7 and -26.9 % compared to controls), no HCD[5] provided in the full study report<br><br>- at 1000 mg/kg bw/d **increased number of pregnant females with implantations only** (0/10, 0/9, 0/9, 7/8 at 0, 100, 300 and 1000 mg/kg bw/day, respectively) Reduced fertility index at 1000 mg/kg bw/d (no stat. analysis on number of pregnant females is available) was considered not adverse by the study author (100, 90, 90, 80 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively). No HCD available.<br><br>F0 females (at 1000 mg/kg bw/day): increased relative and absolute ovary weight (+5.56 %, 16.67 %, 66.67 %** rel. and +5.61 %, 11.21 %, 39.25 %** abs. vs control at 100, 300 and 1000 mg/kg bw/day, respectively), absence of abnormal histological findings in assessed ovaries (in 5/2/1/10 females). As litter loss was only observed at high dose females, it is unlikely to be related to higher ovary weights in all dose groups.<br><br>Other parameters relevant to sexual function and fertility showing no changes vs. controls:<br><br>F0 males/females (up to 1000 mg/kg bw/day): mating index (100 %)<br>F0 males (up to 1000 mg/kg bw/day): testis weight and morphology (3/5 testes tubular bilateral atrophy (grade 1) in HD[5] vs 2/5 in control considered non-adverse); spermatogenesis<br>F0 females (up to 1000 mg/kg bw/day): normal length of oestrous cycle<br><br>F1 generation (up to 300 mg/kg bw/day, $7 \leq n \leq 10$): anogenital distance and anogenital distance index (normalised) on PND 1, nipple retention on PND 13<br><br>**Parental toxicity**:<br>Clinical observations, considered non-adverse:<br>- slight salivation (all treated animals, after dosing)<br>- occasional piloerection (females: 0/10, 1/10, 2/10, 4/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>Effects on food consumption at MD[9] and HD[6], considered non-adverse:<br><br>- occasionally lower food consumption, stat. sign. | |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | ($p < 0.05$), with no effect on the overall mean FC[4] (females: -19.61 % at 300 mg/kg bw/day on LD[7] 7-13; -18.18 % at 1000 mg/kg bw/day on 17-20 d.p.c.[3])<br><br>Effects on body weight and body weight gain at HD, considered non-adverse due to the post-implantation loss of pregnancies in 7 out of 8 HD females (see also Figure 2):<br><br>- lower body weight at the end of gestation, stat. sign. ($p < 0.05$) at 1000 mg/kg bw/day (females: on the 17th d.p.c. 0.0 %, -2.02 %, -7.41 %* and on the 20th d.p.c. -2.08 %, -4.46 %, -19.05 %** at 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>- lower body weight gain at the end of gestation, stat. sign. ($p<0.01$) at 1000 mg/kg bw/day (on the 17th d.p.c. 28 %, 27 %, 28 %, 19 %** and on the 20th d.p.c. 44 %, 41 %, 42 %, 18 %** compared to day 0 p.c.),<br>- lower terminal body weight (not corrected for differences in age and physiological status of females), stat. sign. ($p < 0.01$), (females: -2.37 %, -5.42 %, -14.92 %** at 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>Organ weights and histopathology:<br>**Liver**<br>- increased abs. and rel. liver weight, stat. sign. ($p < 0.01$) at 1000 mg/kg bw/day, without histopathological findings (males: +4.49 %, 12.82 %, 19.02 %** abs. and +3.7 %, 8.15 %, 15.19 %** rel. vs. control at 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>**Adrenal gland**<br>- **increased incidences of minimal inflammatory cell infiltration** at 1000 mg/kg bw/day (females: 0/5, 0/5, 0/5, 5/5 at 0, 100, 300 and 1000 mg/kg bw/day, respectively) in zona fasciculata and zona reticularis of adrenal cortex<br><br>**Thyroid gland**<br>**- increased incidences and severity (up to slight) of thyroid follicular cell hypertrophy starting at 1000 mg/kg bw/d in both sexes:**<br>(males: 2/5 (1 minimal, 1 slight), 2/5 (minimal), 3/5 (1 minimal, 2 slight), 5/5 (1 minimal, 4 slight) at 0, 100, 300 and 1000 mg/kg bw/day, respectively<br><br>females: 3/5, 2/5, 2/5 (all minimal) at 0, 100, 300 and 4/5 (2 minimal, 2 slight) at 1000 mg/kg bw/day, respectively) | |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | **Hormones of the thyroid system**[10]:<br>- **increased TSH in all treated males** (males (median): +136.19 %*, +125.71 %*, +140.00 % and females (mean): +49.31, +47.57, +15.28 % at 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>- total T4 (males (median): +12.09, -12.43, +12.77 % and females (mean) +5.75, -2.3, + 25.67 % at 100, 300 and 1000 mg/kg bw/day, respectively<br><br>Clinical chemistry, statistically significant changes considered non-adverse:<br>F0 males at 1000 mg/kg bw/day: Lower ALP[2] activity (-28.18 %), increased chloride (+3 %)<br>F0 females at 1000 mg/kg bw/day: Lower ALP (-64.23%)/ALAT[1] (-57.13 %) activity and higher potassium (+19.83 %), total protein (+16.7 %), albumin (+26.6 %), sodium (+1.59 %) and calcium (+6.61 %)<br>Haematology:<br>F0 males: No effects<br>F0 females: Lower numbers of neutrophils (-60 %) and MCV[8] (-5.78 %) at 1000 mg/kg bw/day, considered non-adverse due to the differences in the physiological status of females. | |

**[1]ALAT: alanine aminotransferase**

**[2]ALP: alkaline phosphatase**

**[3]d.p.c.: post coitum day**

**[4]FC: Food consumption**

*Fertility index (%) = (Pregnant females / Females mated) x 100*

**[5]HCD: historical control data**

*[6]HD: high-dose*
*[7]LD: lactation day*

**Mating index (%) = (Females mated / Females paired) x 100**

*[8]MCV: mean corpuscular volume*
*[9]MD: mid-dose*
*[10] Statistical analysis was performed by the DS with SigmaPlot software (version 14, Systat Software Inc.). It included a Shapiro-Wilk test for normality and ANOVA on ranks for non-normally distributed data (TSH and T4 in males) followed by Dunnett's post-hoc test for pairwise comparison between groups. For normally distributed data (TSH and T4 in females), a one-way ANOVA test was performed.*

*Historical control data reproduced from the full study report (Charles River, 2018b):*
*"Historical control data for female Wistar Han rats (2015 - 2017):*
- *Total T4 (µg/dL): mean = 3.45, P5 - P95 = 1.700 – 5.060*

*Historical control data for male Wistar Han rats (fasted) (2017 – January 2018):*
- *Alkaline phosphatase (U/L): mean = 163, P5 - P95 = 94 – 284 (n=95)"*

**Table 11: Summary table of human data on adverse effects on sexual function and fertility**

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No data on the effects of 4-phenylbenzophenone on human sexual function and fertility were available | | | | |

**Table 12: Summary table of other studies relevant for toxicity on sexual function and fertility**

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| 10-days<br>Dose-range finding study<br>GLP | 4-phenyl-benzo-phenone, identified as Omnirad 4-PBZ<br>Purity: 99.74 % | No guidelines<br>Crl:WI(Han) rats<br>3 females/dose<br>Dose levels (oral, gavage):<br>500, 1000 mg/kg bw/day<br>Vehicle: Same as in 28-day study, i.e. propylene glycol<br>Duration of exposure:<br>10 consecutive days | No treatment-related effects (based on survival, clinical appearance, body weight and food consumption, macroscopic examination and liver and kidney organ weights) | Appendix 6 in (Charles River, 2018b)<br>Reliability: 4 – only summary is available |

### 10.8.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a combined reproduction and repeated dose toxicity study according to OECD TG 422, Wistar Han rats were exposed to 4-phenylbenzopenone orally via gavage for at least 29 days. The study was conducted up to the limit dose, at dose levels of 0, 100, 300 and 1000 mg/kd bw/day, and according to the GLP standards. The selection of the dose level was done based on the results of the dose range finding study (DRF), where two groups of three females each received 500 and 1000 mg/kd bw/day of 4-phenylbenzophenone respectively. In the DRF study, after 10 days of test item administration, no signs of general toxicity were observed, with assessed parameters staying within the normal range (e.g. clinical signs, body weight, food consumption, liver and kidney organ weight, macroscopic observations).

The duration of exposure in the main study varied between males (29 days) and females, and further on between delivering (up to 56 days: for control, low- and mid-dose groups sacrificed on LD 13-15) and non-delivering females (up to 43 days: for the high-dose group sacrificed on 20 d.p.c.). The exposure of males and females started two weeks before the mating period to cover at least two complete oestrous cycles.

In the key study, no mortality was observed among adult treated animals. Clinical signs were limited to slight salivation after dosing in all treated animals and spontaneous piloerection in treated females. A lower food consumption (-18.18 %) compared to pregnant control females with corresponding lower body weight (-7.41 % to -19.05 %) and body weight gain (+19 % vs +28 % in controls, $p < 0.05$) was observed in females at 1000 mg/kg bw/day from day 17 post coitum. Lower food intake and body weight gain are considered as corresponding to the observed mid-to-late term cessations of pregnancies. Mid-dose females consumed app. 20 % less feed during the second half of lactation period (-19.61 % on LD 7-13), which however did not reflect on their body weight or body weight gain.

The **target organs** identified in this repeated dose toxicity study with 4-phenylbenzophenone were thyroid and adrenal glands. In male rats, a dose-dependent increase in incidences of thyroid hypertrophy was further supported by the increased TSH concentrations in all dose groups, reaching significance in low- and mid-dose groups. Notably, the median TSH value in control males corresponds to the value seen for control adult

males in a study with a different substance available to the DS conducted by the test facility. A 2.3-fold increase in the concentration of the TSH was already noted in low dose animals with similar median TSH levels at the higher dose groups (without a clear dose-related further increase in response). This stimulatory effect on thyroid did not lead to a compensatory increase in circulating levels of T4 and thus is considered to be a toxicologically relevant finding. From another point of view, it may be speculated that the increase in TSH was necessary to compensate for reduced T4, caused by the exposure to the substance. In females, an increase in incidences of follicular cell hypertrophy over the relatively high control levels was observed in high-dose females only. Another histopathological finding that raises concern about the substance-specific toxicity is the increased inflammation in the adrenal cortex in high-dose females. While no evidence of cell necrosis was reported, the induction of an inflammatory response in the endocrine organ is considered adverse. Consequently to these findings, the **NOAEL for parental toxicity was set at 300 mg/kg bw/d** based on the increased incidences of follicular cell hypertrophy in males and females and inflammatory cells infiltration in adrenals in females at 1000 mg/kg bw/d.

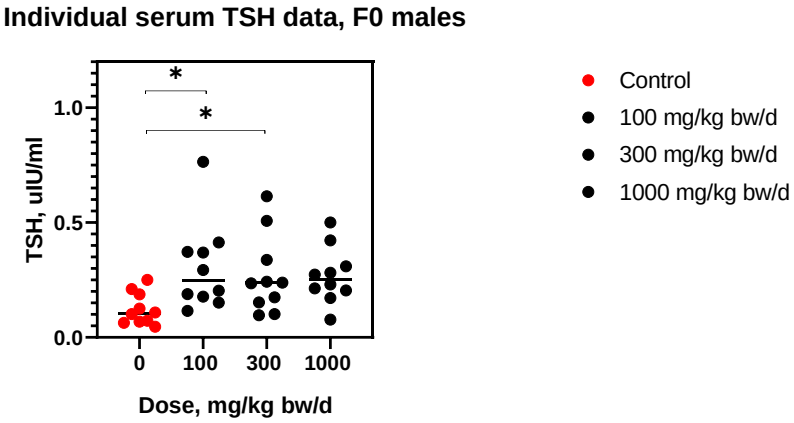



Figure 2: Rats treated with 4-phenylbenzophenone show increased TSH concentration in all treated groups. A scatter plot represents individual values and group medians. Statistical tests were performed using Kruskal-Wallis one way ANOVA on ranks followed by Dunnett's post-hoc test, *p < 0.05.

There were other findings which were observed but judged to be non-adverse: an increase in liver weight in high-dose males (abs. +19.02 % and rel. +15.19 %), without concomitant histological changes in liver tissue and markers of clinical chemistry, and with a lower ALP activity (-28.18 %) of unknown toxicological relevance has been observed. In females, statistically significantly increased ovary weights in high-dose females (abs. +39.25 % and rel. +66.67 %) were considered to be a borderline finding for which a non-ambiguous interpretation is complicated due to the differences in the physiological status between females of the high-dose group vs. the rest of females on the study. In the absence of pathological changes in ovaries (information on follicle numbers and staging is not available), the relevance of the dose-related increase in ovary weights is uncertain.

Exposure to 4-phenylbenzophenone caused **adverse effects on sexual function and fertility.** At 1000 mg/kg bw/d, a **reduced mean number of implantation sites** by 27 % ($p < 0.01$, Steel test) compared to the untreated females was observed. Indeed, the total number of implantation sites as well as number of implantation sites per pregnant dam was altered (see Table 13 below). Furthermore, a drastically high **number of pregnant females with implantations only** reflecting complete litter loss was observed for the females at the same dose level (0/10, 0/9, 0/9, 7/8 at 0, 100, 300 and 1000 mg/kg bw/day, respectively). The latter effect is considered as indication of developmental toxicity and is therefore addressed in the following chapter (see 10.8.8).

**Table 13: Overview on the individual number of implantation sites per dam and on the total number of implantation sites per group**

| | **Dose level, mg/kg bw/day** | | | |
|---|---|---|---|---|
| | **0** | **100** | **300** | **1000** |
| Number of females at the start of the study per group | 10 | 10 | 10 | 10 |
| Pregnant females | 10 | 9 | 9 | 8 |
| Mean number of implantation sites, per pregnant female | 13.0 | 11.8[#] | 12.0 | 9.5 |
| Individual number of implantation sites, per pregnant female | **Females with indicated number of implantation sites** | | | |
| 16 | - | - | 1 | - |
| 14 | 5 | 3 | 1 | - |
| 13 | 2 | 2 | 1 | 2 |
| 12 | 2 | - | 2 | - |
| 11 | - | 2[#] | 2 | 3 |
| 10 | 1 | 1 | 1 | - |
| 9 | - | - | 1 | 1 |
| 7 | - | - | - | 1 |
| 6 | - | 1 | - | - |
| 1 | - | - | - | 1 |
| 0 | - | 1 | 1 | 2 |
| Total number of implantation sites, per group | 130 | 106 (-18.46 %) | 108 (-16.92 %) | 76 (-41.54 %) |

[#]*Mating of one female (No.59) was overlooked and her pregnancy was confirmed due to the living foetuses in the uterus during sacrifice. She had eleven implantation sites and was included in the current calculations.*

The available information on the reduced mean number of implantation sites gives a hint on pre-implantation effects. Pre-implantation loss can be estimated from corpora lutea counts compared to the total implants per female in treated and control groups. In the current study design, corpora lutea were counted only for females with zero implantation sites[3], therefore the evidence of pre-implantation loss cannot be clearly assessed. It remains unclear which molecular mechanism(s) are responsible for the observed adverse effects on female fertility, which may well be caused by the disruption of hormones regulating pregnancy.

Based on the current study and in the absence of other reliable *in vivo* data, the effect on the male reproductive system remains unclear. A very slight increase in the incidences of bilateral testicular atrophy over the background level (3/5 at 1000 mg/kg bw/day vs. 2/5 in control) was not considered adverse by the study authors and by the DS due to a small difference in incidence rate between treated vs. untreated males. On the one hand, no qualitative alterations in spermatogenesis in treated males of all doses were observed. On the other hand, the pre-mating exposure length of two weeks does not cover the complete cycle of rat spermatogenesis. Based on publicly available sources, an androgen receptor (AR) antagonist activity was predicted for the 4-phenylbenzophenone by the COMPARA (Consensus) QSAR Models (EPA-US, 2022). Besides, the CERAPP Potency Level (Consensus) QSAR Model predicted weak estrogenic and anti-estrogenic properties of 4-phenylbenzophenone (EPA-US, 2022). In a screening for interaction with a panel of nuclear receptors, no ER/AR antagonistic properties were demonstrated, however ER1 and ER2 agonistic activities were revealed for 4-phenylbenzophenone (Simon et al., 2016). Thus, effects on male reproductive function cannot be explicitly excluded.

[3] The non-pregnant females from the treated groups had *0 corpora lutea* (0, 1, 1, 2 at 0, 100, 300 and 1000 mg/kg bw/day, respectively).

Overall, **the reproductive NOAEL was set at 300 mg/kg bw/d** (based on reduced number of implantation sites at 1000 mg/kg bw/day).

In conclusion, the GLP- and OECD TG 422-compliant study provides sufficient evidence of adverse effects on sexual function and fertility of treated females.

### 10.8.3 Comparison with the CLP criteria

According to the CLP criteria for Category 1A for known human reproductive toxicants, Annex I: 3.7.2.1.1 of CLP Regulation 1272/2008: "*Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans…. The classification of a substance in this Category 1A is largely based on evidence from humans."*

Data on the effects of 4-phenylbenzophenone on human sexual function and fertility are not available, therefore the criteria for classification to Category 1A for reproductive toxicity are not fulfilled.

Further on, regarding the CLP criteria for Category 1B for presumed human reproductive toxicants, Annex I: 3.7.2.1.1 stipulates: "*The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects*".

In the available reliable GLP-compliant study in rats, conducted according to OECD TG 422 a reduction in **mean number of implantation sites** by 27 % at 1000 mg/kg bw/day in comparison to untreated control females was observed. Furthermore, this effect contributed to the severely compromised pregnancy outcome, with **no live offspring** being delivered by females at 1000 mg/kg bw/day: one out of eight females had two dead pups at first litter check, while seven out of eight pregnant females had implantation sites only, with neither dead nor live offspring. Since a reduction in **mean number of implantation sites** with confirmed pregnancies in the high-dose group represents a clear evidence of adverse effects on sexual function and fertility, the DS considers **classification to Category 1B** as the most appropriate. The observed adverse effects on female fertility occurred together with organ toxicity (thyroid hypertrophy and inflammatory cell infiltration in adrenal gland), but in the absence of general toxicity. Survival, clinical signs, food consumption and changes in body weight or body weight gain did not indicate overt systemic toxicity. Taken together, the evidence of target organ toxicity is not sufficient to conclude that effects on fertility could be a secondary non-specific consequence to other toxic effects.

*"However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate",* according to CLP Regulation 1272/2008, Annex I: 3.7.2.1.1.

No mechanistic information that raises doubt about the relevance of the effect for humans exists, therefore classification in Category 2 is not justified.

*"If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification",* according to CLP Regulation 1272/2008, Annex I: 3.7.2.1.1.

The available combined 28-day repeated dose toxicity study in rats was conducted according to OECD TG 422 in a GLP-certified contract research laboratory. Occasional deviations from the study protocol (five in total) were well-documented. They were not considered by the study author and by the DS to have an impact on the interpretation of the study results and thus do not undermine the reliability of this key study. There were no deficiencies that would make the quality of the available evidence less convincing, thus Category 2 is not justified.

Overall, based on the results of the reliable combined 28-day repeated dose toxicity study, classification as **category 1B for reproductive toxicity based on adverse effects on sexual function and fertility** is warranted for 4-phenylbenzophenone.

### 10.8.4 Adverse effects on development

**Table 14: Summary table of animal studies on adverse effects on development[4]**

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| Combined 28-day repeated dose toxicity study<br><br>OECD TG 422<br><br>GLP<br><br>Male and female Crl:WI(Han) rats<br>(10 sex/dose) | 4-phenylbenzophenone, identified as Omnirad 4-PBZ<br><br>Purity: 99.74 %<br><br>Dose levels (oral, gavage):<br>0, 100, 300, 1000 mg/kg bw/day<br><br>Vehicle: propylene glycol<br><br>Frequency of exposure: once daily, 7 days a week<br><br>Duration of exposure:<br>Males were treated for 29 days, including 14 days prior to mating. Females that delivered were treated for 50-56 days, including 14 days prior to mating and 13-15 days after the delivery. Other females were treated for 39-43 days (1000 mg/kg bw/d) or 41-54 days (100 and 300 mg/kg bw/d). | **Maternal NOAEL** = 300 mg/kg bw/d<br><br>Maternal LOAEL = 1000 mg/kg bw/d (increased incidences of follicular cell hypertrophy in thyroid and inflammatory cell infiltration in adrenals)<br><br>**Developmental NOAEL** < 100 mg/kg bw/d<br>**Developmental LOAEL** = 100 mg/kg bw/d (based on reduced post-implantation survival and decreased live litter size starting at 100 mg/kg bw/d)<br><br>**Developmental toxicity:**<br>- starting at 100 mg/kg bw/d **reduced post-implantation survival index** (89, 82, 78, 3 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively), outside HCD range provided for comparison in the full study report<br><br>- starting at 100 mg/kg bw/d **decreased live litter size** (11.4, 9.8, 9.2, 0 at 0, 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>- at 300 mg/kg bw/d **reduced viability index on PND 4** (100, 99, 95 %, - at 0, 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>- at 300 mg/kg bw/d slightly reduced pup body weight on PND 1 (-7.69 %, p>0.05)<br><br>- at 300 mg/kg bw/d **reduced body weight at PND 7** (-15.72 %) **and PND 13** (-17.49 %), stat. sign. (p<0.05)<br><br>- at 1000 mg/kg bw/d **reduced gestation index** (100, 100, 100, 0 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>- at 1000 mg/kg bw/d **increased number of pregnant females with implantations only** (0/10, 0/9, 0/9, 7/8 at 0, 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>- at 1000 mg/kg bw/d **reduced live birth index** (98, 100, 99, 0 % at 0, 100, 300 and | (Charles River, 2018b)<br><br>**Key study**<br><br>Klimisch Score 1 |

[4] Non-adverse effects are summarised for transparency of reporting. **Adverse effects** are marked in bold to improve understanding of the position of DS.

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | 1000 mg/kg bw/day, respectively).<br><br>**Maternal toxicity**:<br>**100 mg/kg bw/d**: no effects (slight salivation after dosing, considered non-adverse)<br><br>**300 mg/kg bw/d**:<br>Clinical observations: slight salivation after dosing, considered non-adverse<br>Effects on food consumption, considered non-adverse as they were not accompanied by reduced BW (BW gain):<br><br>- lower food consumption on LD 7-13 (-19.61 %), stat. sign. ($p < 0.05$)<br><br>**1000 mg/kg bw/d**:<br><br>Clinical observations, considered non-adverse:<br>- slight salivation after dosing<br>- occasional piloerection (0/10, 1/10, 2/10, 4/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively)<br><br>Effects on food consumption, considered non-adverse:<br><br>- lower food consumption compared to pregnant females, with no effect on the overall mean FC (-18.18 %* on 17-20 d.p.c.)<br><br>Effects on body weight and body weight gain, considered non-adverse as compared to pregnant/older females (see also **Figure 3**):<br>- lower body weight at the end of gestation (-7.41 %* on the 17$^{th}$ d.p.c. and -19.05 %** on the 20$^{th}$ d.p.c.),<br>- lower body weight gain at the end of gestation, (on the 17$^{th}$ d.p.c. 28 %, 27 % , 28 %, 19 %** and on the 20$^{th}$ d.p.c. 44 %, 41 %, 42 %, 18 %** compared to day 0 p.c. at 0, 100, 300 and 1000 mg/kg bw/day, respectively),<br>- lower terminal body weight (-14.92 %**)<br><br>**Adrenal gland- increased incidences of minimal inflammatory cell infiltration** at 1000 mg/kg bw/day (females: 0/5, 0/5, 0/5, 5/5 at 0, 100, 300 and 1000 mg/kg bw/day, respectively) in zona fasciculata and zona reticularis<br><br>**Thyroid** | |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | - **increased incidences and severity (up to slight) of thyroid follicular cell hypertrophy** at 1000 mg/kg bw/d (females: 3/5, 2/5, 2/5 (severity: minimal) at 0, 100, 300 and 4/5 (severity: 2 minimal, 2 slight) at 1000 mg/kg bw/day, respectively) | |

*Gestation index (%) = (Number of females with living pups on Day 1/ Number of pregnant females) x 100*
*Live birth index (%) = (Number of live offspring on Day 1 after littering / Total number of offspring born) x 100*
*Post-implantation survival index (%) = (Total number of offspring born/ Total number of uterine implantation sites) x 100*
*Viability index (%) = (Number of live offspring on Day 4 before culling/ Number live offspring on Day 1 after littering) x 100*
*Lactation index (%) = (Number of live offspring on Day 13 after littering/ Number live offspring on Day 4 (after culling)) x 100*

*Historical control data reproduced unchanged from the full study report (Charles River, 2018b):*

*"Historical control data for female Wistar Han rats (period 2017 - June 2017):*
- *Post-implantation survival index (%): mean = 93 %, P5 - P95 = 84 - 100 (N (studies)=48)*
- *Viability index (%): mean = 98 %, P5 - P95 = 90 - 100 (N=48)"*

**Table 15: Summary table of human data on adverse effects on development**

| Type of data/report | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No data on the effects of 4-phenylbenzophenone on human development were available | | | | |

**Table 16: Summary table of other studies relevant for developmental toxicity**

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other studies on the effects of 4-phenylbenzophenone on mammalian development were available | | | | |

### 10.8.5 Short summary and overall relevance of the provided information on adverse effects on development

In the combined reproduction and repeated dose toxicity study, Wistar Han rats were exposed to 4-phenylbenzophenone orally via gavage for at least 29 days (10 females per dose). The study was conducted up to the limit dose, at dose levels of 0, 100, 300 and 1000 mg/kd bw/day, according to OECD TG 422 (Charles River, 2018b). The dose level was selected based on the results of the dose range finding study where two groups of three females each received 500 and 1000 mg/kd bw/day of 4-phenylbenzophenone respectively. In this study, 10 days after test item administration, no signs of general toxicity were observed, with assessed parameters staying within the normal range (e.g. clinical signs, body weight, food consumption, liver and kidney organ weight, macroscopic observations). In the main study, females were exposed to 4-phenylbenzophenone for two weeks prior to mating and throughout the pregnancy period. Females that did not produce litters were sacrificed on the following day after the expected delivery (39-43 days of exposure in total). The rest of dams were treated until day 13-15 of lactation (54-56 days in total).

**Maternal toxicity** in the main study was limited to thyroid and adrenal histopathological findings in the high-dose group. Reduced food consumption by females at 300 mg/kg bw/day (-19.61 % on LD 7-13) was assessed as non-adverse because it did not affect body weight and body weight gain of females. Taken together, survival, clinical signs, food consumption and changes in body weight or body weight gain did not indicate overt systemic toxicity. However, the pregnancy outcome was severely compromised, with **no live offspring** delivered by females at 1000 mg/kg bw/day: one out of eight females had two dead pups at first litter check, while seven out of eight females had implantation sites only, with neither dead nor live offspring. Consequently to this difference between pregnant vs. non-pregnant females, the body weight of high-dose females on GD 20 was lower by 19.05 % ($p < 0.01$) compared to the body weight of control females at the end of gestation. Though upon delivery of pups on LD1, the body weights of control and high-dose group females were again comparable between each other (see Figure 3).

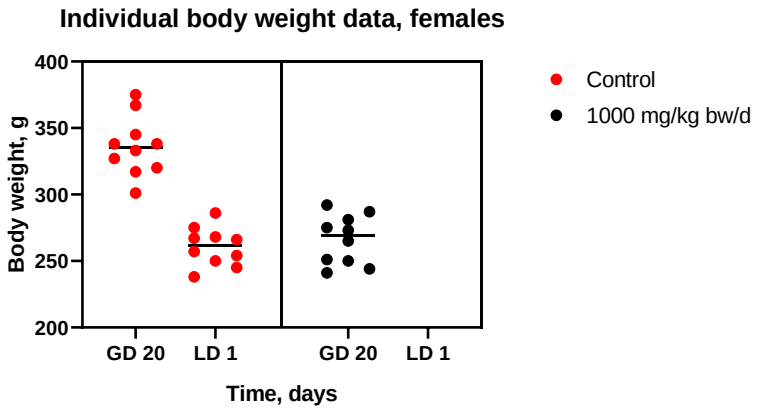



Figure 3: On GD 20 female rats of the high-dose group treated with 4-phenylbenzophenone have comparable body weight to that of the control females after parturition on LD 1. A scatter plot represents individual values and group medians.

**Developmental effects.** A **high number of pregnant females with implantations only** (complete litter loss) was observed for the females at the high-dose group (0/10, 0/9, 0/9, 7/8 at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and is considered to be an adverse effect on development. This effect could have been caused either by the inhibition of blastocysts implantations or by a cessation of development of embryos and intrauterine death. In the case of 4-phenylbenzophenone, both pre- and post-implantation effects take place.

On the one hand, partial inhibition of blastocyst implantation was observed (reduction of implantation sites by 27 % in the high-dose group) and was considered as an adverse effect on sexual function and fertility (see chapter 10.8.2). On the other hand, cessation of development of the embryo is evident from a dose-dependent reduction in post-implantation survival index, which was observed starting at the low dose of 100 mg/kg bw/d, while affecting the high-dose group most severely (89, 82, 78, 3 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively)[5]. Uncertainty remains about whether the finding of seven high-dose dams with *implantation sites only* represents a complete absence of implanted foetuses (following an early post-implantation loss[6] occurring simultaneously in all females) or whether death of developing embryos occurred at the later time point of pregnancy. Histologically there were no further specifications of findings at the implantation sites given. A partial evidence may be represented by a plot with mean body weight data of mated females with confirmed pregnancies. On this plot, a changing slope of the curve of high-dose females during the mid-to-late period of pregnancy may indicate a window of exposure when the pregnancy losses have potentially occurred (see Figure 4).

[5] *HCD for female Wistar Han rats (period 2017 - June2017): mean= 93 %, P5-P95 = 84–100 (N (studies) = 48.*

[6] The post-implantation loss is calculated by determining the ratio of dead to total implants in the treated group compared to the ratio of dead to total implants in the control group.

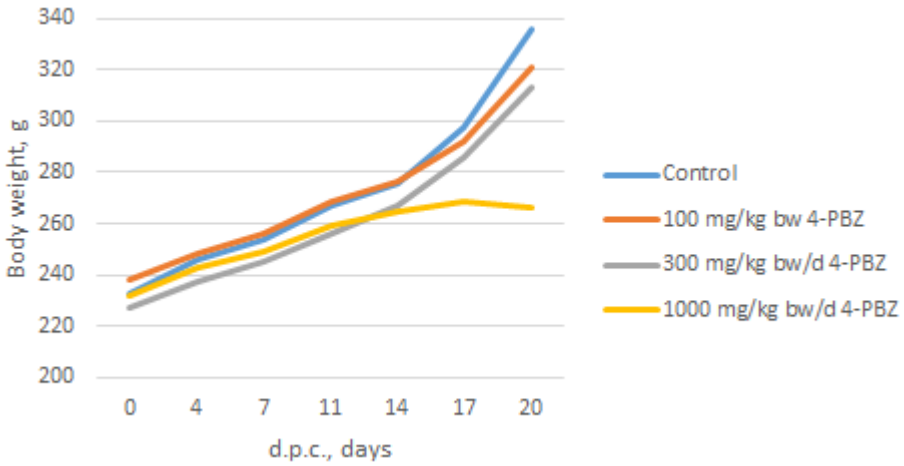



Figure 4. Mean body weight of pregnant female rats treated with 100, 300 and 1000 mg/kg bw/d of 4-phenylbenzophenone over the whole period of pregnancy plotted against the control.

In summary, failed pregnancies at 1000 mg/kg bw/d could potentially be explained by a reduced number of implantation sites (discussed in the chapter above), increased post-implantation loss and still birth (see Table 17 for more details).

For 100 and 300 mg/kg bw/d, a dose-dependent, treatment-related, but statistically not significant reduction in mean litter size was calculated (11.4, 9.8, 9.2, 0 at 0, 100, 300 and 1000 mg/kg bw/day, respectively). Furthermore, post-natal toxicity at 300 mg/kg bw/d was observed, as evidenced by reduced pup viability index (95 % vs. 100 % in controls) and by a statistically significant **reduction in body weight of developing pups** (-15.72 % on PND 7 and -17.49 % on PND 13 vs. control). This could be related to lower food consumption of the dams during LD 7-13 (-19.61 %), however, the lower feed demand could have been caused by a smaller size of litters (-19.3 % vs control) that dams of this group were rearing. Besides, no reduction in food consumption was seen during the mating and gestation periods of this group of females, concurrently the body weight of pups was already slightly affected on the PND 1 (-7.69 %, $p>0.05$). Thus, the causal relationship between lower food consumption in dams and progressive reduction in body weight of developing pups cannot be clearly established (see Table 17).

**Table 17: Table with results of 28-day combined repeated dose toxicity study with 4-phenylbenzophenone in rats according to the OECD TG 422**

| | **HCD** | **Dose level, mg/kg bw/day** | | | |
|---|---|---|---|---|---|
| | | **0** | **100** | **300** | **1000** |
| Total duration of treatment, days | | 56 | 54 | 54 | 43 |
| Exposure prior to mating | | 2 weeks | 2 weeks | 2 weeks | 2 weeks |
| Number of females at the start of the study per group | | 10 | 10 | 10 | 10 |
| Females paired | | 10 | 10 | 10 | 10 |
| Females mated | | 10 | 10 | 10 | 10 |
| Pregnant females | n.a. | 10 | 9 | 9 | 8 |
| | | **Maternal body weight, g** | | | |
| GD 0 | n.a. | 233 | 234 | 227 | 230 |
| GD 14 | n.a. | 275 | 277 | 270 | 269 |
| GD 17 | n.a. | 297 | 297 | 291 | 275* |
| GD 20[§] | n.a. | 336 | 329 | 321 | 272* |
| LD 1 | n.a. | 261 | 260 | 253 | 240[+] |
| LD 7 | n.a. | 282 | 285 | 275 | - |
| LD 13 | n.a. | 303 | 303 | 290 | - |

| | HCD | Dose level, mg/kg bw/day | | | |
|---|---|---|---|---|---|
| | | **Reproductive parameters** | | | |
| Non-gravid females with 0 corpora lutea | n.a. | 0 | 1 | 1 | 2 |
| Mating index, % | n.a. | 100 | 100 | 100 | 100 |
| Fertility index, % | n.a. | 100 | 90 | 90 | 80 |
| Pregnant females with implantations only | n.a. | 0 | 0 | 0 | 7 |
| Females with total litter loss (PND 1) | n.a. | 0 | 0 | 0 | 1 |
| Females with living pups on day 1 | n.a. | 10 | 8[#] | 9 | 0 |
| Total number of uterine implantation sites | n.a. | 130 | 106[#] | 108 | 76 |
| Mean number of implantation sites, per pregnant female | n.a. | 13.0 | 11.8 | 12.0 | 9.5 |
| | | **Developmental parameters** | | | |
| Gestation index, % | | 100 | 100 | 100 | 0 |
| Post-implantation survival index, % | Mean = 93 %, P5-P95 = 84-100, 48 studies | 89 | 82[##] | 78 | 3 |
| Total number of offspring born (incl. stillborn) | n.a. | 116 | 78[##] | 84 | 2 |
| Live birth index, % | n.a. | 98 | 100 | 99 | 0 |
| Number of live offspring on day 1 | n.a. | 114 | 78 | 83 | 0 |
| Number of live offspring on day 4 before culling | n.a. | 114 | 77 | 79 | 0 |
| Live litter size | n.a. | 11.4 | 9.8 | 9.2 | - |
| Viability index on PND 4, % | Mean = 98 %, P5-P95 = 90-100, 48 studies | 100 | 99 | 95 | - |
| Lactation index, % | | 99 | 100 | 100 | - |
| | | **Pup body weight, g** | | | |
| Pups body weight on PND 1 | n.a. | 6.5 | 6.6 (+1.54 %) | 6 (-7.69 %) | - |
| Pups body weight on PND 4 | n.a. | 9.6 | 10 (+4.17 %) | 8.6 (-10.42 %) | - |
| Pups body weight on PND 7 | n.a. | 15.9 | 16.4 (+3.14 %) | 13.4* (-15.72 %) | - |
| Pups body weight on PND 13 | n.a. | 30.3 | 31.1 (+2.64 %) | 25* (-17.49 %) | - |

*p<0.05

[§] *No information on corrected body weight is available*

[+] *This body weight value refers to a single animal because only one dam of the high-dose group delivered pups and was weighed on LD1.*

[#] *One female rat did not mate and mating of another female (No.59) was overlooked and its pregnancy was confirmed due to the living foetuses in the uterus during sacrifice. It had eleven implantation sites.*

[##] *Female No. 59 was excluded from the calculations.*

*n.a. – not available*

*Gestation index (%) = (Number of females with living pups on Day 1/ Number of pregnant females) x 100*

*Live birth index (%) = (Number of live offspring on Day 1 after littering/ Total number of offspring born) x 100*

*Post-implantation survival index (%) = (Total number of offspring born/ Total number of uterine implantation sites) x 100*

*Viability index (%) = (Number of live offspring on Day 4 before culling/ Number of live offspring on Day 1 after*

*littering) x 00*
*Lactation index (%) = (Number of live offspring on Day 13 after littering/ Number live offspring on Day 4 (after culling)) x 100*

In conclusion, the GLP- and OECD TG 422-compliant study provides sufficient evidence of adverse effects on development, such as death of developing embryos (increased post-implantation loss) and delayed postnatal development as evidenced by lower body weight of mid-dose pups on PND 7 and PND 13 (300 mg/kg bw/d). Importantly, no live pups were born in the high-dose group, preventing assessment of the post-natal development of high-dose group pups.

### 10.8.6 Comparison with the CLP criteria

According to the CLP criteria for Category 1A for known human reproductive toxicants, Annex I 3.7.2.1.1 of CLP Regulation 1272/2008: "*Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans…. The classification of a substance in this Category 1A is largely based on evidence from humans."*

No data on the effects of 4-phenylbenzophenone on development in humans were available, therefore the criteria for classification to Category 1A for reproductive toxicity are not fulfilled.

Furthermore, according to the CLP criteria for Category 1B for presumed human reproductive toxicants, Annex I: 3.7.2.1.1 stipulates: "*The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects*".

In the available reliable GLP-compliant study in rats, conducted according to OECD TG 422, a clear evidence of adverse effects on development was observed. The manifestation of the adverse effects on development included **death of developing organisms**: increased **post-implantation loss** (as indicated by females with implantation sites only) and consequential complete litter loss with no live pups born in the high-dose group. Post-implantation survival index was affected in all dose groups (82, 78, 3 %), starting at the low dose, and was below the concurrent control (89 %) and available historical control data (HCD: *mean = 93 %, P5-P95 = 84-100 %, 48 studies*). Live birth outcome was zero at the high-dose as there were only two stillborn pups. Furthermore, the study provides evidence of **altered pup growth** (body weight decrease by 15.72 % at PND 7 and 17.49 % at PND 13 at 300 mg/kg bw/d group vs. control). Growth at the limit dose of 1000 mg/kg bw/d was not assessed due to the absence of live pups from PND 1 onward. Based on these findings, the DS proposes to classify 4-phenylbenzophenone as toxic to reproduction **category 1B**. The adverse effects on development were observed in the absence of severe maternal toxicity, however in the high-dose group of females, thyroid and adrenal glands were identified as target organs. Nevertheless, the DS is of the opinion that the identified toxicities could hardly cause the profound developmental effects observed at this dose level. Besides, developmental toxicity effects in the progeny occurred already at the low and mid doses, thus further supporting the notion of being specific and not a consequence of other toxic effects.

*"However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate"*, according to CLP Regulation 1272/2008, Annex I: 3.7.2.1.1.

No mechanistic information that raises doubt about the relevance of the effect for humans exist therefore classification in Category 2 is not justified.

*"If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification",* according to CLP Regulation 1272/2008, Annex I: 3.7.2.1.1.

The available combined 28-day repeated dose toxicity study in rats was conducted according to OECD TG 422 in a GLP-certified laboratory. Occasional deviations from the study protocol had no impact on the assessment of developmental parameters. There were no deficiencies that would make the quality of the evidence less convincing, thus Category 2 is not justified.

Overall, based on the results of the reliable combined 28-day repeated dose toxicity study, classification as **category 1B for reproductive toxicity based on adverse effects on development** is warranted for 4-phenylbenzophenone.

### 10.8.7 Adverse effects on or via lactation

See below.

### 10.8.8 Short summary and overall relevance of the provided information on effects on or via lactation

In spite of the evidence from the OECD TG 422 study suggesting the involvement of lactation on the development of pups body weight, there are no specific studies with 4-phenylbenzophenone suitable to evaluate effects on or via lactation.

### 10.8.9 Comparison with the CLP criteria

In the absence of experimental data, no comparison with the CLP criteria can be made.

### 10.8.10 Conclusion on classification and labelling for reproductive toxicity

In conclusion, the DS proposes to classify 4-phenylbenzophenone (EC 218-345-2) as Repr. 1B (H360 FD - May damage fertility. May damage the unborn child). As no information on doses below 100 mg/kg bw/d is available, a calculation whether the $ED_{10}$ is 4 mg/kg bw/d and would fulfil the criteria of 3.7.2.6 of the CLP guidance is not possible, thus use of the GCL of 0.3 % (w/v) for the classification of mixtures is proposed.

## 10.9 Specific target organ toxicity-single exposure

Not assessed in this report

## 10.10 Specific target organ toxicity-repeated exposure

Not assessed in this report

## 10.11 Aspiration hazard

Not assessed in this report

# 11 EVALUATION OF ENVIRONMENTAL HAZARDS

## 11.1 Rapid degradability of organic substances

**Table 18: Summary of relevant information on rapid degradability**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| OECD 301 B | 2 % $CO_2$ evolution after 28 days (average of three replicates) | Reliability: 2 (test concentration >> water solubility), GLP (Registrant reliability 1) | Registration dossier (Charles River Laboratories Den Bosch BV, 2017c) |
| BIOWIN (v4.10) | Not readily biodegradable (BIOWIN 3: slower than weeks, BIOWIN 5: < 0.5)<br><br>BIOWIN 1: biodegrades fast (0.8876);<br>BIOWIN 2: biodegrades fast (0.9231);<br>BIOWIN 3: ultimate biodegradation weeks-months (2.6499);<br>BIOWIN 4: primary biodegradation within days or weeks (3.4626);<br>BIOWIN 5: not readily degradable (0.2033);<br>BIOWIN 6: not readily degradable (0.1122) | Reliability: 2 | (BIOWIN v4.11.) |

### 11.1.1 Ready biodegradability

The ready biodegradability of 4-phenylbenzophenone was evaluated in a $CO_2$ Evolution Test according to OECD TG 301 B. The initial concentration of 4-phenylbenzophenone used in this study was 13.5 mg/L. Non-adapted activated sludge from a domestic wastewater treatment plant was used as inoculum (3.6 g/L suspended solids concentration). After 28 days, a biodegradation of 2 % (average of three replicates) was determined. The degradation in the toxicity control reached 41 % after 14 days. The reference compound sodium acetate reached the pass level for ready biodegradability within 9-11 days. The test concentration (13.5 mg/L) was higher than the water solubility (0.0736 mg/L). Therefore, it is possible, that the test substance was not available to the microorganism.

A prediction of the ready biodegradability of 4-phenylbenzophenone supports the result of the experimental study. Based on BIOWIN (see Table 18) the substance is predicted to be not readily biodegradable.

### 11.1.2 $BOD_5$/COD

No data available.

### 11.1.3 Hydrolysis

No data available. 4-Phenylbenzophenone is poorly water-soluble.

### 11.1.4 Other convincing scientific evidence

No data available.

#### 11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

#### 11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

#### 11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

No data available.

#### 11.1.4.4 Photochemical degradation

No data available.

### 11.2 Environmental fate and other relevant information

The adsorption potential was assessed according to OECD TG 121 (HPLC method, GLP) in compliance with GLP (Reliability 1) (Charles River Laboratories Den Bosch BV, 2018b). At neutral pH and 35 °C a log $K_{OC}$ of 4.57 was determined.

### 11.3 Bioaccumulation

**Table 19: Summary of relevant information on bioaccumulation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| OECD 117 | Log Kow = 4.7 (at 35 °C) | Reliability 1 | Registration dossier (Charles River Laboratories Den Bosch BV, 2017b) |

### 11.3.1 Estimated bioaccumulation

No relevant data available.

### 11.3.2 Measured partition coefficient and bioaccumulation test data

The registrant performed a study according to OECD 117 (HPLC-method) to determine the log $K_{OW}$. The log $K_{OW}$ is determined to be 4.7 at 35 °C.

### 11.4 Acute aquatic hazard

**Table 20: Summary of relevant information on acute aquatic toxicity**

| Method | Species | Test material | Results | Remarks | Reference |
|---|---|---|---|---|---|
| OECD TG 203 | *Cyprinus carpio* | CAS 2128-93-0 | 96h-$LC_{50}$ > 0.058 mg/L (meas., arith.mean) | Reliability 1 | Registration dossier (Charles River Laboratories Den Bosch BV, 2017a) |
| OECD TG 202 | *Daphnia magna* | CAS 2128-93-0 | 48h-$EC_{50}$ > 0.069 mg/L (meas., initial) | Reliability 1 | Registration dossier (Charles River Laboratories Den Bosch BV, 2018a) |
| OECD TG 201 | *Raphidocelis subcapitata* | CAS 2128-93-0 | 72h-$E_rC_{50}$ = 0.041 mg/L (meas., TWA) | Reliability 1 | Registration dossier (Charles River Laboratories Den Bosch BV, 2018c) |

### 11.4.1 Acute (short-term) toxicity to fish

A 96-hour acute fish toxicity test according to OECD TG 203 was conducted with *Cyprinus carpio* as a limit test under semi-static conditions. The test concentration nominally 100 mg/L was analytically monitored and no vehicle was used. The measured concentration was 0.058 mg/L. Seven organism per test vessel with one replicate was used. No effects were observed (no mortality). Therefore, the 96h-$LC_{50}$ is higher than 0.058 mg/L (meas., arith.mean).

### 11.4.2 Acute (short-term) toxicity to aquatic invertebrates

One acute toxicity test on the aquatic invertebrate *Daphnia magna* was conducted over 48 hours under static test conditions as a limit test. The test concentration was nominally 100 mg/L and initially measured 0.069 mg/L. No *Daphnia* was immobilised. Therefore, the 48h-$EC_{50}$ is higher than 0.069 mg/L (meas., initial).

### 11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

A 72-hour toxicity test with the aquatic algae *Raphidocelis subcapitata* (previous names: *Pseudokirchneriella subcapitata, Selenastrum capricornutum*) under static test conditions is available. The test was conducted under static test conditions with an initial cell density of 10000 cells/mL and three replicates per test concentrations or six replicates per control). M2 medium was used. The time-weighted average test concentrations were 6.6, 12, 23, 26, 34, and 40 µg/L. All validity criteria according to OECD TG 201 were fulfilled. The resulting 72-hour $E_rC_{50}$ is 0.041 mg/L (measured).

### 11.4.4 Acute (short-term) toxicity to other aquatic organisms

Not available.

## 11.5 Long-term aquatic hazard

**Table 21: Summary of relevant information on chronic aquatic toxicity**

| Method | Species | Test material | Results | Remarks | Reference |
|---|---|---|---|---|---|
| OECD TG 201 | *Raphidocelis subcapitata* | CAS 2128-93-0 | 72h-$E_rC_{10}$ = 0.033 mg/L (meas., TWA)<br>72h-$NOE_rC$ = 0.012 mg/L (meas., TWA) | Reliability 1 | Registration dossier (Charles River Laboratories Den Bosch BV, 2018c) |

### 11.5.1 Chronic toxicity to fish

Not available.

### 11.5.2 Chronic toxicity to aquatic invertebrates

Not available.

### 11.5.3 Chronic toxicity to algae or other aquatic plants

The test is described in section 11.4.3. The results relevant for the evaluation of the chronic toxicity of the substance to algae are: 72h-$E_rC_{10}$ = 0.033 mg/L (measured), 72-h-$NOE_rC$ < 0.0066 mg/L (measured; statistically significant) or 0.012 mg/L (measured, biologically relevant).

### 11.5.4 Chronic toxicity to other aquatic organisms

Not available.

## 11.6 Comparison with the CLP criteria

### 11.6.1 Acute aquatic hazard

**Table 22: Comparison with criteria for acute aquatic hazards**

| | Criteria for acute environmental hazards | 4-phenylbenzophenone | Conclusion |
|---|---|---|---|
| Acute Aquatic Toxicity | Cat. 1: $LC_{50}/EC_{50}/ErC_{50} \leq 1$ mg/L | Fish: 96h-$LC_{50}$ > 0.058 mg/L (meas., arith.mean)<br>Aquatic invertebrates: 48h-$EC_{50}$ > 0.069 mg/L (meas., initial)<br>Algae: 72h-$E_rC_{50}$ = 0.041 mg/L (meas., TWA) | **Aquatic Acute 1, M=10**<br>Based on algae toxicity |

### 11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

**Table 23: Comparison with criteria for long-term aquatic hazards**

| | Criteria for environmental hazards | 4-phenylbenzophenone | Conclusion |
|---|---|---|---|
| Rapid Degradation | Half-life hydrolysis < 16 days<br><br>Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % theoretical oxygen demand, theoretical carbon dioxide) | no data available<br><br>2 % biodegradation after 28 days → not readily biodegradable | **Not rapidly degradable** |
| Bioaccumulation | Log Kow ≥ 4<br>BCF ≥ 500 | Log Kow = 4.7<br>BCF: no data available | **High potential for bioaccumulation** |
| Aquatic Toxicity | Non-rapidly degradable substances:<br>Cat. 1: NOEC ≤ 0.1 mg/L<br>Cat. 2: NOEC ≤ 1 mg/L<br>(based on Table 4.1.0 (b) (i) of the CLP Regulation)<br><br><u>Surrogate approach in absence of appropriate chronic toxicity reference data</u> (based on Table 4.1.0 (b) (iii) of the CLP Regulation)<u>:</u><br>Not rapidly degradable substances and/or bioaccumulative substances:<br>Cat. 1: $E/LC_{50} \leq 1$ mg/L<br>Cat. 2: $E/LC_{50}$ > 1 to ≤ 10 mg/L<br>Cat. 3: $E/LC_{50}$ > 10 to ≤ 100 mg/L | Algae: 72h-$E_rC_{10}$ = 0.033 mg/L (meas., TWA)<br><br>No long-term toxicity data for aquatic invertebrates or fish available.<br><br>Fish: 96h-$LC_{50}$ > 0.058 mg/L (meas., arith.mean)<br><br>Aquatic invertebrates: 48h-$EC_{50}$ > 0.069 mg/L (meas., initial) | **Aquatic Chronic 1, M=1**<br>Based on chronic algae toxicity |

## 11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute aquatic hazard:

The valid $E/LC_{50}$ values from the short-term toxicity tests on fish and aquatic invertebrates are > the maximal water solubility of the substance (nominally 100 mg/L; measured 0.058 or 0.069 mg/L.

The valid $E_rC_{50}$ from the algae toxicity test is 0.041 mg/L and therefore < 1 mg/L. The proposed acute aquatic classification is Aquatic Acute 1 (H400) with an M-factor of 10 based on the criteria given in Table 4.1.0 (a) and Table 4.1.3 of the CLP Regulation.

Chronic aquatic hazard:

4-Phenylbenzophenone is not rapidly degradable and has a high potential for bioaccumulation in the aquatic environment, as the log Kow is higher than 4 (4.7).

Chronic toxicity data is available only for algae. The valid long-term toxicity value is the $E_rC_{10}$ of 0.033 mg/L (meas., TWA). This results in a classification of 4-phenylbenzophenone as Aquatic Chronic 1 (M= 1) based on the criteria given in Table 4.1.0 (b) (i) and Table 4.1.3 of the CLP Regulation.

For the other two trophic levels, the surrogate approach based on Table 4.1.0 (b) (iii) of the CLP Regulation has to be used. As no effects occurred up to the maximum achievable water solubility, no classification based on these results is justified.

The most stringent outcome of the two assessments according to Table 4.1.0 (b) (i) and (iii) results in a classification of 4-phenylbenzophenone as Aquatic Chronic 1 (H410) with a M-factor of 1 (based on Table 4.1.0 (b) (i) and Table 4.1.3 of the CLP Regulation).

## 12 EVALUATION OF ADDITIONAL HAZARDS

**Not assessed in this report**

## 13 ADDITIONAL LABELLING

Not assessed in this report

## 14 REFERENCES

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