Committee for Risk Assessment

RAC

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

Adopted

30 November 2023

30 November 2023

CLH-O-0000007379-62-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **30 November 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **4-phenylbenzophenone**

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

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

Rapporteur, appointed by RAC: **Marieta Fernandez**

Co-Rapporteur, appointed by RAC: **Benjamin Piña**

Administrative information on the opinion

Germany on **22 February 2023** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **27 March 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **26 May 2023**.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H360FD H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H360FD H317 H410		 M = 10 M = 1	
RAC opinion	TBD	4-phenylbenzophenone	218-345-2	2128-93-0	Repr. 1B Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H360FD H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H360FD H317 H410		 M = 10 M = 1	
Resulting Annex VI entry if agreed by COM	TBD	4-phenylbenzophenone	218-345-2	2128-93-0	Repr. 1B Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H360FD H317 H400 H410	GHS08 GHS07 GHS09 Dgr	H360FD H317 H410		 M = 10 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance 4-phenylbenzophenone [also known as 4-benzoylbiphenyl; biphenyl-4-yl(phenyl)methanone], EC 218-345-2, CAS 2128-93-0, does not currently have an Annex VI entry. It 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. Similarly, PubChem database reports its use in/as paints and coatings, falling into the category of "consumer uses", according to the US EPA. However, according to ECHA's substance dissemination webpage (Substance Infocard), no consumer use has been reported by EU notifiers. Despite this, there is growing concern about its use in food packaging and subsequent migration into food products (Chen et al., 2022), as well as in in e-waste contaminated dust (Li et al., 2020).

Based on its physico-chemical characteristics, 4-phenylbenzophenone is lipophilic (log P_{ow} = 4.7 at 35 °C, pH 7) and poorly water-soluble (0.0736 mg/L at 20°C, pH 6.5). Its volatility is low, with vapour pressure values below or equal to 1.9×10^{-5} Pa at 20 °C and below or equal 4.3×10^{-5} Pa at 25 °C. Based on its molecular weight of 258.32 g/mol, the low water solubility and the log P_{ow} of 4.7, limited oral and dermal absorption would be expected. However, the Lipinski's rule of five [Danish (Q)SAR Database], predicted high oral bioavailability, with an 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.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

There was one ***in vivo*** **animal study** included in the CLH report; a reliable GLP-compliant **local lymph node assay (LLNA)** in mouse (strain: CBA:J) which was carried out according to OECD TG 429, with a deviation of maximum tested concentrations (25% instead of 50%), due to higher concentrations showing poor solubility (Charles River, 2018a). A dose-dependent increase in local lymphocyte proliferation was observed, showing that 4-phenylbenzophenone has skin sensitising properties. At the highest concentration tested (25% w/w), a stimulation index (SI) value of 3 (rounded) [2.9 (mean) and 3.8 (median), (EC3 > 2%)] was reported, suggesting that 4-phenylbenzophenone is a skin sensitiser of moderate potency.

***Table**: Summary of the LLNA study*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results**	**Reference**
Local lymph node assay (LLNA) OECD TG 429 GLP Reliability: 1	Female CBA:J mice, (5/group)	4-phenylbenzophen-one, identified as Omnirad 4-PBZ Batch: 20161118 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 Positive control was not included for scientific and animal welfare reasons.	**Positive** Mean SI values (at test concentrations): 1.3 (5%) 2.3 (10%) 2.9 (25%)	(Charles River, 2018a)

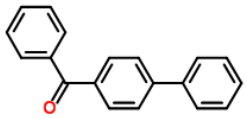
There was also **one *in vitro* assay** study discussed in the CLH report; the **KeratinoSensTM assay** (Charles River, 2017b). In that assay, 4-phenylbenzophenone showed a positive 1.5-fold induction at the concentration of 0.64 µM and a 1.7-fold maximal induction of the antioxidant/electrophile response element (ARE)-response pathway, while no cytotoxicity was observed at this dose level. This *in vitro* assay supports the classification of 4-phenylbenzophenone as a skin sensitiser in a weight-of-evidence evaluation. The 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 (2nd 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.

In addition, there was **one *in silico* prediction report** discussed in the CLH report, using the Derek Nexus (v.5.0.2) knowledge based QSAR system, employing the Derek KB 2015 2.0 knowledge database (Charles River, 2017a). The prediction generated by Derek Nexus is based on the training set containing similar substances and predicted that 4-phenylbenzophenone did not contain any of the 80 alerts for skin sensitisation known to the system. It should be noted that the most recent OECD TG 497 on defined approaches for skin sensitisation calls for the use of the most recent version of Derek software (v.6.1.0) for the prediction of skin sensitising properties.

There was also **one *in chemico* skin sensitisation: direct peptide reactivity assay (DPRA)** discussed in the CLH report, which investigated the potential of binding of the 4-phenylbenzophenone to cysteine- and lysine-containing synthetic peptides (Charles River, 2017c). Low cysteine and lysine reactivity was predicted, contradicting the *in vivo* findings, possibly due to precipitation of 4-phenylbenzophenone upon addition to solution with the synthetic peptides containing lysine (SPCL). OECD TG 442C recommends caution in interpreting negative results if precipitates are observed (OECD, 2021b).

***Table**: Summary of other studies relevant for skin sensitisation (Table 9 in the CLH dossier)*

Type of study/data	**Test substance, batch, purity**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
In vitro skin sensitisation: ARE-Nrf2 luciferase test KeratinoSens™ assay OECD TG 442D GLP* Reliability: 1	4-phenylbenzophenone, identified as Omnirad 4-PBZ Batch: 20161118 Purity: 99.74%	Omnirad 4-PBZ in dimethyl sulfoxide; 2 concentration ranges: 1st Exp.: 0.06 – 125 µM 2nd Exp.: 0.0005 – 1 µM KeratinoSens™ cells with ARE-Nrf2 reporter were incubated with respective concentrations of Omnirad 4-PBZ for 48 h. Positive control: Ethylene dimethacrylate glycol Read-out for ARE-pathway activation: Luciferase-based luminescence Cell viability: MTT assay, spectrophotometry, absorption at 570 nm	KeratinoSens™ assay: **positive** Induction of ARE: 1st experiment (0.06-125 µM): $EC_{1.5}$ < 0.06 µM and I_{max} = 18.84, cytotoxicity observed (IC_{30} = 37 µM and IC_{50} = 52 µM) 2nd experiment (0.0005-1 µM): $EC_{1.5}$ = 0.64 µM and I_{max} = 1.7 at cell viability > 70% Positive control: acceptable 1st experiment: $EC_{1.5}$ = 24 µM and I_{max} = 3.7 2nd experiment: $EC_{1.5}$ = 39 µM and I_{max} = 2.53 Variation: acceptable (CV < 20% in 1st and 2nd experiments in DMSO neg. ctrl.)	(Charles River, 2017b)
In chemico skin sensitisation: direct peptide reactivity assay (DPRA) OECD TG 442C GLP Reliability: 1 (GLP certificate was provided in the study report. However, due to the main limitation of the experiment (precipitation), the negative	4-phenylbenzophenone, identified as Omnirad 4-PBZ Batch: 20161118 Purity: 99.74%	100 mM Omnirad 4-PBZ diluted in acetonitrile; incubated for 24 h at 25oC with synthetic peptides SPCC or SPCL. After incubation, the remaining concentrations of peptides were determined using HPLC-PDA.	DRPA prediction: **negative** Reactivity class: no or minimal reactivity class Mean of SPCC and SPCL depletion: 1.5% at 100 mM SPCC depletion: 0.6±1.1% (mean ±SD) SPCL depletion: 2.5±2.7% (mean ±SD) Limitation: test item precipitation, limiting the amount of test item in the solution required for interaction with the SPCL peptide	(Charles River, 2017c)

results should be interpreted with caution).				
In silico skin sensitisation: QSAR Derek prediction on skin sensitisation GLP not applicable	4-phenylbenzophenone CAS Number: 2128-93-0, Molecular weight: 258.3, Molecular formula: C19H14O Structure: 	Derek Nexus ver. 5.0.2, Nexus 2.1.1 Knowledge Database: Derek KB 2015 2.0	QSAR prediction: **negative** No alerts for skin sensitisation	(Charles River, 2017a)

ARE: antioxidant/electrophile responsive element
SPCC: synthetic peptides containing cysteine
SPCL: synthetic peptides containing lysine
HPLC-PDA: high-performance liquid chromatography with gradient elution and photodiode array detection

It should be noted that no human data on the effects of 4-phenylbenzophenone as a skin sensitiser were available.

Based on the reliable GLP-compliant LLNA study, considered a key study relevant for classification, the DS proposed to classify 4-phenylbenzophenone 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.

Comments received during consultation

One Member State Competent Authority (MSCA) provided a comment stating that they generally agree with the proposed classification (however, indicating support for Skin Sens. 1A, while the proposal is for Skin Sens. 1B) based on the 25% effective concentration inducing an SI of 3 (rounded) in the LLNA test (EC3 > 2%).

Assessment and comparison with the classification criteria

According to the classification criteria for skin sensitisers, Annex I: 3.4.2.2.1.1, CLP Regulation:

"*Skin sensitizers shall be classified in Category 1 where data are not sufficient for sub-categorisation.*"

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

Classification in sub-category 1A is warranted if the criteria given in Annex I, Table 3.4.2 of the CLP Regulation are fulfilled: *"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".

There were no human data available to assess frequency of occurrence or severity of skin sensitisation reactions to 4-phenylbenzophenone. In the LLNA in accordance with OECD TG 429, the EC_3 value was higher than 2% (the limit concentration below which are defining the threshold for skin sensitisers of high potency according to Annex I, Table 3.4.3 of the CLP Regulation). Hence, classification in sub-category 1A is considered not applicable.

Classification as skin sensitisers in sub-category 1B is warranted when the criteria stated in Annex I, Table 3.4.2 of the CLP Regulation are fulfilled: *"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, there were no human data available for 4-phenylbenzophenone. The LLNA demonstrated moderate potency of 4-phenylbenzophenone, with an EC_3 value of 25% at the highest achieved tested concentration (above 2% threshold in Annex I, Table 3.4.4 of the CLP Regulation).

RAC concludes that **classification as Skin Sens. 1B; H317 (May cause an allergic skin reaction) for 4-phenylbenzophenone is warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The CLH dossier included one study relevant to this endpoint, a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test according to OECD TG 422, in which Wistar Han rats were exposed 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/kg bw/d, and according to the GLP standards. The duration of exposure varied between males (29 days) and females (delivering females up to 56 days, and non-delivering females up to 43 days (high dose) or 41-54 days (low and mid dose)). The exposure of males and females started two weeks before the mating period to cover at least two complete oestrous cycles.

Survival, clinical signs, food consumption and changes in body weight or body weight gain did not indicate overt systemic toxicity.

In male rats, a dose-dependent increase in incidences of thyroid hypertrophy, supported by increased thyroid stimulating hormone (TSH) concentrations in all dose groups (without a compensatory increase in circulating levels of thyroxine (T4)) was observed, reaching significance in low- and mid dose groups. In females, an increase in incidences of follicular cell hypertrophy was also observed, but only in the high dose group. In high dose females, increased inflammation in the adrenal cortex was also observed. The NOAEL for parental toxicity was set at 300 mg/kg bw/d.

Exposure to 4-phenylbenzophenone caused adverse effects on sexual function and fertility. At the high dose, a reduced mean number of implantation sites by 27% ($p < 0.01$, Steel test) was observed; the total number of implantation sites as well as the number of implantation sites per pregnant dam was altered. The reproductive NOAEL was set at *300 mg/kg bw/d.*

***Table**: Individual number of implantation sites per dam and the total number of implantation sites per group (Table 13 in the CLH dossier)*

	Dose level, mg/kg bw/d			
	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
Total number of implantation sites, per group	130	106 (-18.46%)	108 (-16.92%)	76 (-41.54%)

#Mating of one female 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.

***Table**: Summary table of the adverse effects on sexual function and fertility (Table 10 in the CLH dossier)*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test Male and female Crl:WI(Han) rats (10/ sex/dose) OECD TG 422 GLP Klimisch score 1	4-phenylbenzophenone, identified as Omnirad 4-PBZ Purity: 99.74% Dose levels (oral, gavage): 0, 100 (LD), 300 (MD), 1000 (HD) mg/kg bw/d Vehicle: Propylene glycol Frequency of exposure: Once daily, 7 days a week Duration of exposure: 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 (HD) or 41-54 days (LD and MD).	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 HD) Reproductive NOAEL = 300 mg/kg bw/d (based on reduced number of implantation sites at HD) **Reproductive toxicity**: 1000 mg/kg bw/d: - **reduced mean number of implantation sites** (13.0, 11.8, 12.0, 9.5** in control, LD, MD and HD, respectively corresponding to -9.2, -7.7 and -26.9% compared to controls). No HCD provided in the full study report. - **increased number of pregnant females with implantations only** (0/10, 0/9, 0/9, 7/8 in control, LD, MD and HD, respectively) - **reduced fertility index**: 100, 90, 90, 80% in control, LD, MD and HD, respectively (no stat. analysis on number of pregnant females is available; considered not adverse by the study author). No HCD available. F0 females (at HD): - increased relative and absolute	(Charles River, 2018b) Key study

		ovary weight (relative: +5.56%, 16.67%, 66.67%** and absolute: +5.61%, 11.21%, 39.25%** compared to controls at LD, MD and HD, respectively) - no abnormal histological findings in assessed ovaries As litter loss was only observed in high dose females, it is unlikely to be related to higher ovary weights, which was seen in all dose groups. **Other parameters related to sexual function and fertility showing no changes compared to controls**: F0 males/females (up to 1000 mg/kg bw/d): - no effects on mating index (100%) F0 males (up to 1000 mg/kg bw/d): - no treatment related effects on testis weight and morphology (3/5 testes tubular bilateral atrophy (grade 1) in HD vs 2/5 in control considered non-adverse) - no effects on spermatogenesis F0 females (up to 1000 mg/kg bw/d): - no effects on length of oestrous cycle F1 generation (up to 300 mg/kg bw/d): - no effects on anogenital distance and anogenital distance index (normalised) on PND 1 - no effects on nipple retention on PND 13 **Parental toxicity**: Clinical observations, considered non-adverse: - slight salivation (all treated animals, after dosing) - occasional piloerection (females: 0/10, 1/10, 2/10, 4/10 in control, LD, MD and HD, respectively) Effects on food consumption at MD and HD, considered non-adverse: - occasionally lower FC, with no effect on the overall mean FC (females: -19.61% at MD on lactation day 7-13; -18.18% at HD on 17-20 d.p.c.) 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 high dose females: - lower body weight at the end of gestation (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%** in control, LD, MD and HD, respectively) - lower body weight gain at the end of gestation (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.), - lower terminal body weight (not corrected for differences in age and physiological status: females: -2.37%, -5.42%, -14.92%** at LD, MD and HD, respectively <u>Organ weights and histopathology</u>: *Liver:* - increased absolute and relative liver weight, without histopathological findings (males: absolute: +4.49%, 12.82%, 19.02%** and relative: +3.7%, 8.15%, 15.19%** compared to controls at LD, MD and HD, respectively) *Adrenal gland:* - increased incidences of minimal inflammatory cell infiltration at 1000 mg/kg bw/d (females: 0/5, 0/5, 0/5, 5/5 in control, LD, MD and HD, respectively) in *Zona fasciculata* and *Zona reticularis* of adrenal cortex *Thyroid gland*: Increased incidences and severity (up to slight) of thyroid follicular cell hypertrophy in both sexes: - males: 2/5 (1 minimal, 1 slight), 2/5 (minimal), 3/5 (1 minimal, 2 slight), 5/5 (1 minimal, 4 slight) in control, LD, MD and HD, respectively - females: 3/5, 2/5, 2/5 (all minimal) in control, LD and MD, respectively, and 4/5 (2 minimal, 2 slight) at HD *Thyroid hormones*: Increased TSH (compared to controls at LD, MD and HD, respectively): - males (median): +136.19%*, +125.71%*, +140.00% - females (mean): +49.31%, +47.57%, +15.28% Total T4 (compared to controls at 100, 300 and 1000 mg/kg bw/d, respectively): - males (median): +12.09%, -12.43%, +12.77% - females (mean) +5.75%, -2.3%, + 25.67% *Clinical chemistry* (statistically significant changes, considered non-adverse): <u>1000 mg/kg bw/d:</u> F0 males: - lower ALP activity (-28.18%), increased chloride (+3%) F0 females: - lower ALP (-64.23%)/ALAT (-57.13%) activity and higher potassium (+19.83%), total protein (+16.7%), albumin (+26.6%), sodium (+1.59%) and calcium	

		(+6.61%) *Haematology:* F0 males: - no effects F0 females: - lower numbers of neutrophils (-60%) and MCV (-5.78%) at HD, considered non-adverse due to the differences in the physiological status of females.	

* statistically significant, $p < 0.05$; ** statistically significant, $p < 0.01$
ALAT: alanine aminotransferase; ALP: alkaline phosphatase; d.p.c.: post coitum day; FC: Food consumption; Fertility index (%) = (Pregnant females / Females mated) × 100; HCD: historical control data; PND: postnatal day; Mating index (%) = (Females mated / Females paired) × 100; MCV: mean corpuscular volume; LD: low dose; MD: mid dose; HD: high dose;

A very slight increase in the incidences of bilateral testicular atrophy above background level (3/5 at 1000 mg/kg bw/d vs. 2/5 in control) was not considered adverse. The pre-mating exposure length of two weeks in the study did not cover the complete cycle of rat spermatogenesis; hence, the effect on the male reproductive system has not been completely assessed.

Since the reliable OECD TG 422 study is considered a key study relevant for the classification, the DS proposed to classify 4-phenylbenzophenone for adverse effects on sexual function and fertility in Category 1B.

Adverse effects on development

In the reliable OECD TG 422 study, females were exposed to 4-phenylbenzophenone for two weeks prior to mating and throughout the pregnancy period. No signs of general toxicity were observed, and the maternal toxicity was limited to thyroid and adrenal histopathological findings in the high dose group. However, the pregnancy outcome was severely compromised, with no live offspring delivered by females at 1000 mg/kg bw/d (mean live litter size/dam was 11.4, 9.8, 9.2, 0 and post implantation survival index was 89, 82, 78 and 3%, at 0, 100, 300 and 1000 mg/kg bw/d, respectively). Since no live pups were born in the high dose group, assessment of the post-natal development of high dose group pups could not be performed. Furthermore, a 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 dams at 0, 100, 300 and 1000 mg/kg bw/d, respectively). Post-natal toxicity at 300 mg/kg bw/d was observed with reduced pup viability index (95% vs. 100% in controls) and statistically significant reduction in body weight of developing pups (-15.72% on PND 7 and -17.49% on PND 13 vs. control).

The DS considered that there is sufficient evidence of adverse effects on development, such as death of developing embryos (increased post-implantation loss, total litter loss and no live pups born at 1000 mg/kg bw/d) and delayed postnatal development as evidenced by lower body weight of mid dose pups on PND 7 and PND 13. The adverse effects on development were observed in the absence of severe maternal toxicity. In the high dose females, thyroid and adrenal glands were affected (see table with summary of study results). However, the DS was of the opinion that the identified effects did not cause the severe developmental effects observed at this dose level. In addition, some effects on development in the progeny occurred already at the low and mid dose, thus further supporting the effects being specific and not a non-specific consequence of other toxic effects. Based on the clear effects seen in the OECD TG 422 study, the DS proposed to classify 4-phenylbenzophenone for adverse effects on development in Category 1B.

Adverse effects on or via lactation

There are no specific studies with 4-phenylbenzophenone available to evaluate adverse effects on or via lactation.

In conclusion, the DS proposed to classify 4-phenylbenzophenone as Repr. 1B; H360FD (May damage fertility and the unborn child).

Comments received during consultation

Three comments were received, two from MSCAs (supporting the proposed Repr. 1B; H360FD classification), and one from a National Authority.

One MSCA proposed that genotoxicity (not assessed) could be the mode of action (MoA) for the observed effects on fertility and development. The same MSCA commented that although no severe maternal toxicity was observed, the thyroid and adrenal glands were identified as target organs in the high dose females and that this could point to an endocrine disrupting effect. The DS responded that it would have been interesting to assess it, but that a comparative MoA analysis is not required for the purpose of a CLH classification for adverse effects on reproduction.

The National Authority commented that in the absence of a full corpora lutea count, it is difficult to determine whether the reduction in implantation sites was a result of a decrease in the number of eggs produced or a failure of implanted blastocyst progression (questioning the support for Category 1B for adverse effects on sexual function and fertility). The DS agreed that studies covering the effects of 4-phenylbenzophenone in early pregnancy, e.g., using the decidual cell response technique, could have been useful. Similarly, analysing possible cytotoxic effects on the germ cell cycle or on the proliferating cells of blastocysts could have been of use. The DS, however, responded that early pregnancy loss is viewed as a manifestation of reduced fertility and that their proposal to classify as Repr. 1B for adverse effects on sexual function and fertility is considered justified.

Assessment and comparison with the classification criteria

The criteria to assess and compare the classification criteria are provided in Annex I, 3.7.2.1.1 of CLP Regulation[1].

[1] According to the criteria for Category 1A (known human reproductive toxicants), Annex I, 3.7.2.1.1 of CLP Regulation: *"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."*
According to the CLP criteria for Category 1B (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*":

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

Adverse effects on sexual function and fertility

No data on the effects of 4-phenylbenzophenone on sexual function and fertility in humans were available; therefore, the criteria for classification in Category 1A for are not fulfilled.

One GLP-compliant study in rats, conducted according to OECD TG 422, was available. In that study, exposure to 4-phenylbenzophenone caused clear adverse effects on sexual function and fertility. At 1000 mg/kg bw/d, the average number of implantation sites was reduced by 27%; the total number of implantation sites as well as the number of implantation sites per pregnant dam was altered.

During the consultation, one MSCA commented that there was no full corpora lutea count, and that it was difficult to determine whether the reduction in implantation sites was a result of a decrease in the number of eggs produced or a failure of implanted blastocyst progression. Due to this, the MSCA questioned the support for Category 1B for adverse effects on sexual function and fertility. RAC noted, however, that the reduction in implantation sites is an effect on sexual function and fertility regardless of whether it was caused by a decrease in number of eggs or a failure of implanted blastocyst progression.

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

The available OECD TG 422 study in rats was conducted in a GLP-certified laboratory. According to RAC, occasional deviations from the study protocol had no impact on the assessment of fertility parameters. There were no deficiencies that would make the quality of the evidence less convincing; thus, Category 2 is not justified.

Based on the effects on female reproduction, RAC concluded that classification for adverse effects on sexual function and fertility in Category 1B (Repr. 1B; H360F) is warranted for 4-phenylbenzophenone.

Adverse effects on development

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

In the available reliable GLP-compliant OECD TG 422 study in rats, 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 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 (Charles River, 2018b[2]). 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%

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

[2] Historical control data, reproduced unchanged from the full study report (Charles River, 2018b): 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)"

at PND 13 at 300 mg/kg bw/d group compared to control). Post-natal effects at 1000 mg/kg bw/d was not assessed due to the absence of live pups from PND 1 onward.

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

The available OECD TG 422 study in rats was conducted 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.

Based on the results of the reliable combined 28-day repeated dose toxicity study, RAC concluded that classification for adverse effects on development in Category 1B (Repr. 1B; H360D) is warranted for 4-phenylbenzophenone.

As no information on doses below 100 mg/kg bw/d was available, a calculation of whether the ED_{10} is below 4 mg/kg bw/d and would fulfil the criteria for assigning a specific concentration limit (ECHA Guidance on the Application of the CLP Criteria, 2017, 3.7.2.6) is not possible; thus, use of the GCL of 0.3% (w/v) for the classification of mixtures is proposed.

No classification for adverse effects on or via lactation is proposed due to lack of data.

Overall, RAC agreed with the DS and concluded that **4-phenylbenzophenone warrants classification as Repr. 1B; H360FD (May damage fertility and the unborn child).**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

The ready biodegradability of 4-phenylbenzophenone was evaluated in a CO_2 Evolution Test according to OECD TG 301B. 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. This is supported by a prediction of the ready biodegradability of 4-phenylbenzophenone using BIOWIN that also predicted that the substance is not readily biodegradable.

These results indicate that 4-phenylbenzophenone is not readily/rapidly biodegradable.

Bioaccumulation

The DS performed a study according to OECD TG 117 (HPLC-method) to determine the log K_{OW}. The log K_{OW} is determined to be 4.7 at 35°C. This value indicates that 4-phenylbenzophenone is bioaccumulative (log K_{OW} > 4). In the absence of experimental BCF values, the log K_{OW} criterion should be used for classification.

Aquatic toxicity

Acute aquatic toxicity

***Table**: relevant acute aquatic toxicity data*

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)

The valid E/LC_{50} values from the short-term toxicity tests on fish and aquatic invertebrates are above 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, below 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 toxicity

***Table**: relevant chronic aquatic toxicity data*

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) 72h NOE_rC = 0.012 mg/L (meas., TWA)	Reliability 1	Registration dossier (Charles River Laboratories Den Bosch BV, 2018c)

4-phenylbenzophenone is not rapidly degradable and has a high potential for bioaccumulation in the aquatic environment, as the log K_{OW} is higher than 4 (4.7).

Chronic aquatic toxicity data is available only for algae. The only available, 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 CLP Annex I, 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 warranted.

Thus, the DS considered that the most stringent outcome of the two assessments according to CLP Annex I, 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).

Comments received during consultation

Two comments were received, one from a National Authority and one from an MSCA. Both agreed with the proposal, but one asked for a confirmation of the statistical methodology and the possible use of further study/data on bioaccumulation. In both cases it was agreed that the requested information could only affect M-factors but not the classification category; RAC noted that a potential refinement of the M-factor value could take place once the new information will become available.

Assessment and comparison with the classification criteria

RAC agreed with the DS that the substance is **not rapidly degradable** based on the outcome of the OECD TG 301B study and a supporting QSAR estimation.

RAC agreed with the DS that the substance has a **potential to bioaccumulate** based on the log K_{OW} value of 4.7.

RAC concluded that the substance warrants an aquatic acute classification as **Aquatic Acute 1**, with an **acute M-factor of 10**, based on the E_rC_{50} value of 0.041 mg/L for *Raphidocelis subcapitata* (in agreement with the DS).

Table: *Comparison with criteria for acute aquatic hazards*

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

RAC concluded that the substance warrants **an aquatic chronic classification as Aquatic Chronic 1**, as the most stringent outcome of the assessment according to CLP Annex I, Table 4.1.0 (b) (i) and (iii) (in agreement with the DS). An associated **chronic M-factor of 1** must be set based on Table 4.1.3 of the CLP Regulation.

***Table**: Comparison with criteria for chronic aquatic hazards*

	Criteria for chronic aquatic hazard	4-phenylbenzophenone	Conclusion
Rapid Degradation	Half-life hydrolysis < 16 days Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % theoretical oxygen demand, theoretical carbon dioxide)	no data available 2 % biodegradation after 28 days => not readily biodegradable	**Not rapidly degradable**
Bioaccumulation	Log K_{ow} ≥ 4 BCF ≥ 500	Log K_{ow} = 4.7 BCF: no data available	**High potential for bioaccumulation**
Aquatic Toxicity	Non-rapidly degradable substances: Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L (based on Table 4.1.0 (b) (i) of the CLP Regulation) <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> Not rapidly degradable substances and/or bioaccumulative substances: Cat. 1: E/LC_{50} ≤ 1 mg/L Cat. 2: E/LC_{50} > 1 to ≤ 10 mg/L Cat. 3: E/LC_{50} > 10 to ≤ 100 mg/L	Algae: 72h-E_rC_{10} = 0.033 mg/L (meas., TWA) No long-term toxicity data for aquatic invertebrates or fish available. Fish: 96h-LC_{50} > 0.058 mg/L (meas., arith.mean) Aquatic invertebrates: 48h-EC_{50} > 0.069 mg/L (meas., initial)	**Aquatic Chronic 1, M=1** Based on chronic algae toxicity, namely based on the comparison with the CLP Annex I, Table 4.1.0 (b)(i) criteria. Using the surrogate approach (comparison with CLP Annex I, Table 4.1.0 (b)(iii) criteria) does not lead to a classification, in the presence of only unbounded values for acute fish and aquatic invertebrates.

Additional references

Chen, M. L., Chen, C. H., Huang, Y. F., Chen, H. C., & Chang, J. W. (2022). Cumulative Dietary Risk Assessment of Benzophenone-Type Photoinitiators from Packaged Foodstuffs. Foods, 11(2). https://doi.org/10.3390/foods11020152

Li, J., Li, W., Gao, X., Liu, L., Shen, M., Chen, H., Zhu, M., Zeng, L., & Zeng, E. Y. (2020). Occurrence of multiple classes of emerging photoinitiators in indoor dust from E-waste recycling facilities and adjacent communities in South China and implications for human exposure. Environment International, 136. https://doi.org/10.1016/J.ENVINT.2020.105462

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter and additional information (if applicable)

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).