Committee for Risk Assessment

RAC

Annex 1

Background document

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

Dibenzoyl peroxide; benzoyl peroxide

EC Number: 202-327-6
CAS Number: 94-36-0

CLH-O-0000007215-78-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

1 December 2022

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBENZOYL PEROXIDE; BENZOYL PEROXIDE

CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: Dibenzoyl peroxide; benzoyl peroxide

EC Number: **202-327-6**

CAS Number: **94-36-0**

Index Number: **617-008-00-0**

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Version number: 2.0 **Date: 23/11/2021**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBENZOYL PEROXIDE; BENZOYL PEROXIDE

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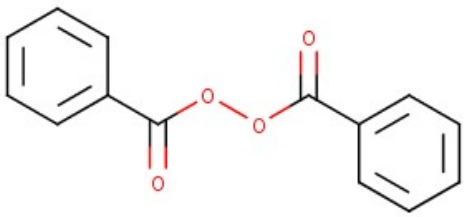
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBENZOYL PEROXIDE; BENZOYL PEROXIDE

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)	Dibenzoyl peroxide; benzoyl peroxide
Other names (usual name, trade name, abbreviation)	Benzoyl peroxide; Diphenylperoxyanhydride; Methanone, 1,1'-dioxybis[1-phenyl-; Benzoic acid, peroxide; Benzoperoxide; Benzoyl superoxide;
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	202-327-6
EC name (if available and appropriate)	Dibenzoyl peroxide
CAS number (if available)	94-36-0
Other identity code (if available)	617-008-00-0
Molecular formula	$C_{14}H_{10}O_4$
Structural formula	
SMILES notation (if available)	O=C(OOC(=O)C1=CC=CC=C1)C1=CC=CC=C1
Molecular weight or molecular weight range	242.227g
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	>=73.5 - <=76.5 % (w/w)

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)
Dibenzoyl peroxide EC No. 202-327-6 CAS No. 94-36-0	Mono-constituent substance	Org. Perox. B H241 Eye Irrit. 2 H319 Skin Sens. 1 H317	Org. Perox. B H241 Eye Irrit. 2 H319 Skin Sens. 1 H317 Aquatic Acute 1 H400 (M factor=10) Aquatic Chronic 1 H410 (M factor=10)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

No impurities relevant for classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

No additives relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

	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	617-008-00-0	Dibenzoyl peroxide; benzoyl peroxide	202-327-6	94-36-0	Org. Perox. B Eye Irrit. 2 Skin Sens. 1	H241 H319 H317	GHS02 GHS01 GHS07 Dgr	H241 H319 H317	-	-	-
Dossier submitters proposal					**Add** Aquatic Acute 1 Aquatic Chronic 1	**Add** H400 H410	**Add** GHS09	**Add** H410	-	**Add** M=10 M=10	-
Resulting Annex VI entry if agreed by RAC and COM					Org. Perox. B Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H241 H319 H317 H400 H410	GHS02 GHS01 GHS07 GHS09 Dgr	H241 H319 H317 H410	-	M=10 M=10	-

Table 6: 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		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity		
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure		
Aspiration hazard		
Hazardous to the aquatic environment	Harmonised classification proposed: Aquatic Acute 1 (M factor=10) Aquatic Chronic 1 (M factor=10)	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Dibenzoyl peroxide was classified under the Dangerous Substance Directive (DSD) 67/548/EEC for physical (R3 and R7) and human health hazards (R36 and R43) and included in Annex I of the DSD in accordance with the 30th Adaptation to Technical Progress (ATP) (Commission Directive 2008/58/EC). The harmonised classification was translated to the CLP Regulation (EC) No. 1272/2008, as Organic Peroxide B H241, Eye Irritant Category 2 H319, and Skin Sensitisation Category 1 H317, and included in Annex VI Table 3.2 of the CLP Regulation (EC) No. 1272/2008 by the 1st ATP (Commission Regulation (EC) No 790/2009).

The current entry in Annex VI of CLP of dibenzoyl peroxide does not include harmonised classification for environmental hazards (i.e. hazardous to the aquatic environment). This proposal intends to update the current harmonised classification entry by including Aquatic Acute Category 1 (M factor=10) and Aquatic Chronic Category 1 (M factor=10).

RAC general comment
Dibenzoyl peroxide is an organic peroxide in the form of a granular powder (crystal) and is used in polymerisation reactions (polymers, resins, rubbers) and as an intermediate, adhesive, sealant, coating resin hardener, and toner by industrial and professional workers. It is also formulated into fillers, adhesives, sealants, cosmetics, and personal care products for use by consumers.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Change in existing entry due to new data for environmental endpoints
Differences in self-classification among the C&L Inventory notifiers with respect to classification for environmental hazards (1701 notifiers and 26 aggregated notifications).

5 IDENTIFIED USES

According to the REACH Registration dossier (ECHA, 2021a), dibenzoyl peroxide is an organic peroxide in the form of a granular powder (crystal) and is used in polymerisation reactions (polymers, resins, rubbers) and as an intermediate, adhesive, sealant, coating resin hardener, and toner by industrial and professional

workers. It is also formulated into fillers, adhesives, sealants, cosmetics and personal care products for use by consumers (ECHA, 2021a).

6 DATA SOURCES

Data for dibenzoyl peroxide is taken from:

- Publically disseminated REACH registration dossier (ECHA, 2021a).
- Publically available literature.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid (white crystalline powder)	ECHA, 2021a	Measured
Melting/freezing point	106°C at 101.3 kPa	ECHA, 2021a	Available literature
Boiling point	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with column 2 of Annex VII of REACH.
Relative density	1.33 at 25°C	ECHA, 2021a	Measured
Vapour pressure	0.009 Pa at 25°C	ECHA, 2021a	Estimated
Surface tension	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with column 2 of Annex VII of REACH.
Water solubility	0.35 mg/L at 20ºC	ECHA, 2021a	Measured Note: dibenzoyl peroxide undergoes rapid abiotic degradation. Benzoic acid is the main degradation product and has a water solubility of 2.9 g/L at 20ºC.
Partition coefficient n-octanol/water	Log Kow: 3.2 at 22°C	ECHA, 2021a	Measured
Flash point	Not applicable	ECHA, 2021a	The registration dossier provided an adaptation in accordance with column 2 of Annex VII of REACH.
Flammability	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with column 2 of Annex VII of

Property	Value	Reference	Comment (e.g. measured or estimated)
			REACH.
Explosive properties	Explosive	ECHA, 2021a	Dibenzoyl peroxide is classified as Organic Peroxide Type B (reference CLP regulations 2.15.1.2, 2.15.2.2 and UN Recommendations on the Transport of Dangerous Goods, 16th revised edition, section 2.5.3.2.4).
Self-ignition temperature	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with column 2 of Annex VII of REACH.
Oxidising properties	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with column 2 of Annex VII of REACH.
Granulometry	135µm (wet powder)	ECHA, 2021a	Measured as a wet powder.
Stability in organic solvents and identity of relevant degradation products	Not applicable	ECHA, 2021a	Only required if the stability of the substance is considered critical.
Dissociation constant	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with section 1 of Annex XI of REACH.
Viscosity	No data	ECHA, 2021a	The registration dossier provided an adaptation in accordance with section 1 of Annex XI of REACH.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated as part of this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated as part of this dossier.

10 EVALUATION OF HEALTH HAZARDS

Not evaluated as part of this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 8: Summary of relevant information on rapid degradability

Method	Test Material	Results	Remarks	Reference
OECD 301 D (Ready Biodegradability: Closed Bottle Test)	Dibenzoyl peroxide Purity: 74.3%	Readily biodegradable. 71% degradation of test substance after 28 days (% degradation (O_2 consumption)). 10-day window pass level requirement achieved.	Key study. Reliability score 1 (reliable without restriction). GLP compliant. No deviations.	Anonymous, 2015b. ECHA Dissemination site, 2021.
OECD 301 D (Ready Biodegradability: Closed Bottle Test)	Dibenzoyl peroxide Purity: 74.6%	Inherently biodegradable. 68% degradation of test substance after 28 days (% degradation (O_2 consumption)). 10-day window pass level requirement not achieved.	Supporting study. Reliability score 2 (reliable with restriction). GLP compliant. The inoculum cell density (10^7 to 10^8 cells/litre) was higher than recommended by the OECD 301 Test Guideline (10^4 to 10^6 cells/litre).	Anonymous, 2009c. ECHA Dissemination site, 2021.
OECD 301 D (Ready Biodegradability: Closed Bottle Test)	Dibenzoyl peroxide Purity: 74.4%	Not readily biodegradable. 56% degradation of test substance after 28 days (% degradation (O_2 consumption)).	Supporting study. Reliability score 2 (reliable with restriction). Deviations from the test guideline are reported. It is unclear if these deviations affected the biodegradation potential of the test material or validity of the study. Refer to Section 11.1.1 and Annex I of the CLH report for further study details.	Anonymous, 1990. ECHA Dissemination site, 2021.

Method	Test Material	Results	Remarks	Reference
			GLP compliant.	
OECD 301 C (Ready Biodegradability: Modified MITI Test (I))	Dibenzoyl peroxide Purity: information not available	Readily biodegradable. 88% degradation of the test substance after 21 days (% degradation (TOC removal)).	Not enough data available to evaluate the validity of the study. Reliability score 4 (not assignable).	Anonymous, 1992. ECHA Dissemination site, 2021.

11.1.1 Ready biodegradability

The ready biodegradability of dibenzoyl peroxide was evaluated in four ready biodegradability studies in accordance with international standards or accepted guidelines. The reliability scores assigned to the studies range between 1 and 4 (Klimisch H.J., Andreae M., and Tillmann U, 1997).

The ready biodegradability of dibenzoyl peroxide was evaluated in a valid GLP compliant OECD Ready Biodegradability closed bottle test (OECD 301 D) (Anonymous, 2015b). Secondary activated sludge (non-adapted, 0.4 g (DW)/L pre-conditioned), obtained from the Nieuwgraaf wastewater treatment plant in Duiven (The Netherlands), was exposed to 2 mg/L dibenzoyl peroxide for 28 days. The test temperature reportedly ranged between 22-24°C. Under the test conditions, dibenzoyl peroxide reported a theoretical oxygen demand (ThOD) of 2.7 mg/L, corresponding to 71% degradation after 28 days. The 10-day window pass level was achieved with over 60% biodegradation reported in a period of approximately 10 days immediately following the attainment of 10% biodegradation. The dossier submitter considers the validity criteria of the test, as stipulated in the OECD 301 guideline, fulfilled. The reference substance, sodium acetate, reached 91% degradation after 14 days and 1.2 mg/L endogenous respiration at Day 28. The residual oxygen concentration remained above 0.5 mg/L in all test bottles over the duration of the test. The dossier submitter considers that under the conditions of the study, dibenzoyl peroxide can be considered readily biodegradable.

In a second GLP OECD 301 D (Anonymous, 2009c) test, activated sludge (concentration equivalent to a maximum of 30 mg/L, pre-conditioned, non-adapted), prepared in the laboratory from secondary effluent from a wastewater treatment plant with activated sludge treating domestic wastewater in the municipality of Abidos (France), was exposed to 4 mg/L dibenzoyl peroxide for 28 days. The oxygen depletion in the inoculum blank did not exceed 1.5 mg/L after 28 days. However, the residual oxygen concentration remained above 0.5 mg/L. The reference substance, sodium benzoate, reached 42% degradation by Day 14 which is below the validity threshold of ≥ 60%. The validity criteria of the test, as stipulated in the OECD 301 guideline, were not fulfilled. Under the conditions of the study, dibenzoyl peroxide biodegraded 68% by Day 28, exceeding the 60% threshold for this test system. However, this level of biodegradation was not

achieved within the 10-day window. The dossier submitter considers that, under the conditions of the study, dibenzoyl peroxide demonstrated inherent biodegradability.

In a further GLP compliant OECD 301 D (Anonymous, 1990) test, the ready biodegradability of dibenzoyl peroxide, 1.5 mg/L (applied using silica gel), was investigated in secondary activated sludge (non-adapted, pre-conditioned) from an activated sludge plant predominantly treating domestic wastewater in Duiven, The Netherlands. After 28 days, dibenzoyl peroxide reportedly degraded by 56%. The test was extended to 84 days, however, the level of degradation did not exceed 56%. The reference substance, sodium acetate, biodegraded by 88 and 94 % by Day 15 and 28, respectively. The study summary reports that inhibitory effects of dibenzoyl peroxide on the micro-organisms of the inoculum were not observed. Considering the extent of degradation, the dossier submitters considers that dibenzoyl peroxide did not fulfil the ready biodegradability criteria. Under the conditions of the study, it was concluded that dibenzoyl peroxide can be considered as not readily biodegradable. The dossier submitter notes that although the validity criteria of the OECD 301 guideline were fulfilled the following deviations were noted (please see Annex I for further study details): secondary activated sludge was used as the inoculum instead of the recommended secondary effluent (or surface water) as per the OECD 301 guideline. The OECD 301 D guideline recommends for substances with a water solubility below 1 g/L that stock solutions are prepared in mineral medium or added directly to the mineral medium rather than in water/solvent as was performed in the study. A test concentration of 1.5 mg/L dibenzoyl peroxide was used in the study. In accordance with the general test conditions reported in Table 2 of the OECD 301 guideline, a test concentration of between 2-10 mg/L is recommended for the OECD 301 D test system.

The ready bioavailability of dibenzoyl peroxide was evaluated in accordance with an OECD Ready Biodegradability modified MITI test (OECD 301 C) (Anonymous, 1992) for 21 days. Under the conditions of the study, dibenzoyl peroxide reported 88% degradation after 21 days. The robust study summary concluded that dibenzoyl peroxide was readily biodegradable, however, no further information is reported in the study summary for the dossier submitter to verify the validity of the study. The study is not considered reliable by the dossier submitter.

The dossier submitter notes that benzoic acid (EC No. 200-618-2) is considered the main degradation product of dibenzoyl peroxide. The robust study summary for benzoic acid, as per the REACH registration dossier, reports that benzoic acid can be considered as readily biodegradable (ECHA dissemination site, 2021b).

11.1.2 BOD_5/COD

No relevant information available.

11.1.3 Hydrolysis

Table 9: Summary of relevant information on hydrolysis

Method	Test Material	Results	Remarks	Reference
OECD 111	Dibenzoyl peroxide Purity: 74.6%	Dibenzoyl peroxide was determined to be hydrolytically unstable at pH 4, 7 and 9 at 50°C. Half-life (DT_{50}): $T_{1/2}$ (pH 4): <1 day at 25°C; $T_{1/2}$ (pH 7): <1 day at 25°C; $T_{1/2}$ (pH 9): <1 day at 25°C. Degradation product: benzoic acid.	Key study. GLP compliant. Reliability score 1 (reliable without restriction).	Anonymous, 2010d. ECHA Dissemination site, 2021.
OECD 111	Dibenzoyl peroxide Purity: 70%	Half-life (DT_{50}): $T_{1/2}$ (pH 4): 11.9 hr at 25°C; Type: (pseudo-) first order (half-life) $T_{1/2}$ (pH 7): 5.2 hr at 25°C; Type: (pseudo-)first order (half-life) $T_{1/2}$ (pH 9): not detected.	Supporting information. Non-GLP. Not enough data available to evaluate the validity of the study. Reliability score 4 (not assignable).	Anonymous, 2001c. ECHA Dissemination site, 2021.

In a GLP compliant OECD Hydrolysis as a Function of pH (OECD 111) study (Anonymous, 2010d), dibenzoyl peroxide was determined to be hydrolytically unstable at acidic (pH 4), neutral (pH 7) and alkaline (pH 9) conditions at 50°C. The rate of hydrolysis increased with increasing pH and resulted in the availability of approximately 20% of the applied test substance to measure at pH 9 on initial sampling. The corresponding first order hydrolysis rate constant was determined. The robust study summary reports that greater than 50% hydrolysis occurred after 2.4 hours, equivalent to a half-life (DT_{50}) of less than 1 day under environmentally relevant condition (25°C). Hydrolysed samples were analysed at each test pH under modified HPLC conditions. The principal hydrolysis product, benzoic acid, was detected in the hydrolysed solutions that were sampled at each tested pH. The dossier submitter notes that the available data for benzoic acid, as reported in the REACH registration dossier (ECHA, 2021b), suggests that benzoic acid can be considered as readily biodegradable and has a low bioaccumulation potential (log Kow < 2). Benzoic acid has a harmonised classification as Skin Irritant 2, Eye Damage 1, and STOT RE 1 (Index number 607-705-00-8). Benzoic acid does not have a harmonised classification entry for environmental hazards.

The hydrolytic stability of non-radiolabelled dibenzoyl peroxide was investigated in a preliminary non-GLP compliant study in accordance with OECD 111 (Anonymous, 2001c). The robust study summary indicates

that, under the conditions of the study, dibenzoyl peroxide degraded 93.5, 94.1 and 94.2% by Day 5 at pH 4, 7 and 9 and at 50 ºC, respectively. The half-life of dibenzoyl peroxide at pH 4 and 7 was determined to be 11.9 hours and 5.2 hours at 25°C, respectively, while the half-life at pH 9, at 25°C, could not be determined as dibenzoyl peroxide was not detected. However, no further information is reported in the robust study summary for the dossier submitter to verify the validity of the study or the observed results. The study is not considered reliable by the dossier submitter.

Based on the available information, the dossier submitter considers dibenzoyl to be hydrolytically unstable at pH 4, 7 and 9. The DT_{50} (25°C) is estimated to be < 1 day. The robust study summary indicates that no additional testing was performed.

11.1.4 Other convincing scientific evidence

Using EPISuite (HENRYWIN v3.20), the dossier submitter calculated a Henry's Law constant of dibenzoyl peroxide of 3.54 x 10^{-6} Pa m³/mol, suggesting little potential for dibenzoyl peroxide to volatilise in the environment. The registration dossier reports that dibenzoyl peroxide has a vapour pressure of 9 x 10^{-3} Pa at 25°C and can be considered as non-volatile. Substances with a Henry Law constant below 3.04 x 10^{-2} Pa m³/mol are less volatile than water and can be considered essentially non-volatile.

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

Not relevant.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available or required. Please refer to section 11.1.1.

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

No data available.

11.1.4.4 Photochemical degradation

An atmospheric half-life of 3.009 days for dibenzoyl peroxide was calculated, by the dossier submitter, using EPISuite (AOPWIN v1.92).

11.1.5 Summary and discussion on environmental degradation.

Abiotic degradation: Dibenzoyl peroxide was determined to be hydrolytically unstable at pH 4, 7 and 9 with an estimated DT_{50} (25°C) of < 1 day. In addition, dibenzoyl peroxide has a Henry Law constant value (calculated) of 3.54 x 10^{-6} Pa m³/mol and is considered to be non-volatile.

Biotic degradation: the readily biodegradability of dibenzoyl peroxide was tested in accordance with three OECD 301D and one OECD 301C ready biodegradability studies. The rate of degradation ranged from between 56 to 88%. In accordance with Annex I: 4.1.2.9.5 of the CLP Regulation (EC) No. 1272/2008,

'Substances are considered rapidly degradable in the environment if one of the following criteria holds true (a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved: (i) tests based on dissolved organic carbon: 70 %; (ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.'. In conclusion, based upon the available information (as presented in Table 11 and section 11.1.1), the dossier submitter considers dibenzoyl peroxide to be rapidly degradable in the environment.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.3 Environmental fate and other relevant information

No further data.

11.4 Bioaccumulation

Table 10: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
EPISuite KOWWIN and BCFBAF	log Kow 3.43 BCF = 89.11 L/kg	-	EPISuite, 2021.
OECD 121	log Koc 3.8 at 22°C Purity: 74.6%	Key study. GLP compliant. No deviations. Reliability score of 1 (reliable without restriction).	Anonymous, 2009a. ECHA dissemination site, 2021.
OECD 117	log Kow 3.2 at 22°C Purity: 74.6%	Key study. GLP compliant. No deviations. Reliability score of 1 (reliable without restriction).	Anonymous, 2009b. ECHA dissemination site, 2021.

11.4.1 Estimated bioaccumulation

The EPISuite KOWWIN (v1.68) and BCFBAF QSAR models predict a log Kow and BCF value for dibenzoyl peroxide of 3.43 (log Kow 3.43) and 89.11 L/kg, respectively.

11.4.2 Measured partition coefficient and bioaccumulation test data

The partition coefficient of dibenzoyl peroxide was determined experimentally in a GLP compliant OECD Partition Coefficient (n-octanol) study (OECD 117) which reported a partition coefficient value of 3.2 (log Kow 3.2) at 22°C (Anonymous, 2009a). Aquatic bioaccumulation studies to determine the bioconcentration of dibenzoyl peroxide in aquatic species are not available.

The adsorption/desorption behaviour of dibenzoyl peroxide on soil and sewage sludge was evaluated in a GLP compliant OECD Estimation of the Adsorption Coefficient on Soil and on Sewage Sludge study using High Performance Liquid Chromatography (OECD 121) (Anonymous, 2009a). An organic carbon-water partition coefficient value (Koc) of 6310 for dibenzoyl peroxide was reported corresponding to a log Koc of 3.8 at 22°C. As per Section R.7.9.4.3 of ECHA's Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b, substances with log Koc > 4 are generally regarded as highly adsorptive and likely to distribute in sediment and soil. Dibenzoyl peroxide can be considered moderately-to-highly adsorptive.

According to the CLP Regulation (EC) No. 1272/2008, "*a cut-off value of log Kow ≥ 4 is intended to identify only those substances with a real potential to bioconcentrate…A BCF in fish of ≥ 500 is indicative of the potential to bioconcentrate for classification purposes*". For classification purposes, the log Kow value is used when an experimentally determined BCF is not available.

As there are no experimental BCF studies reported in the registration dossier, the bioaccumulation potential of dibenzoyl peroxide was determined by comparing the experimentally derived log Kow value with the CLP cut-off criteria. Considering this, dibenzoyl peroxide is considered to have a low bioaccumulation potential based on the experimentally derived log Kow of 3.2, which is less than the CLP cut-off value of ≥ 4. Furthermore, the QSAR calculated log Kow (3.43) and BCF value of dibenzoyl peroxide (89.11 L/kg) are less than the CLP cut-off criteria (log Kow ≥ 4; BCF ≥ 500).

The dossier submitter considers dibenzoyl peroxide to have a low bioconcentration potential and is not considered bioaccumulative for classification purposes.

11.5 Acute aquatic hazard

Table 11: Summary of relevant information on acute aquatic toxicity

Fish					
Method	**Species**	**Test material**	**Results**	**Remarks**	**Reference**
OECD 203; EU Method C.1	*Oncorhynchus mykiss* (Rainbow trout)	Dibenzoyl peroxide Purity: 74.6%	LC_{50} (96 hr, semi-static): 0.0602 mg/L (mean measured)	Key study. GLP compliant. No deviations	Anonymous, 2010a. ECHA dissemination

		Test concentrations (mg/L): Nominal: 0.427, 0.939, 2.07, 4.55 and 10. Mean measured: 0.0081, 0.0097, 0.0224, 0.0316 and 0.0741.		reported. Reliability score of 1 (reliable without restriction).	site, 2021.
OECD 203; EU Method C.1	*Poecilia reticulata* (Guppy)	Dibenzoyl peroxide Purity: 74.4% Test concentrations (mg/L): Nominal: 0.7, 1.3, 2.4 and 4.2.	LC_{50} (96 hr, aerated semi-static): 2 mg/L (nom.)	Supporting study. GLP compliant. Analytical monitoring was not performed. Test organisms obtained from local aquarium retailer. The validity criteria of the study are not fulfilled. Reliability score of 3 (not reliable).	Anonymous, 1989. ECHA dissemination site, 2021.
OECD 203; EU Method C.1	*Oryzias latipes* (Japanese medaka)	Dibenzoyl peroxide Purity: 97.3% Test concentrations (mg/L): Nominal: 0.25, 0.5, 1.0, 2.0 and 4. Mean measured: 0.23, 0.47, 0.69, 1.54 and 2.17.	LC_{50} (96 hr, flow-through): 0.24 mg/L (mean measured)	Supporting study. GLP compliant. Source of test species unknown. Length of test species deviated from guideline. The robust study summary indicates the	Anonymous, 2002. ECHA dissemination site, 2021.

				study is of secondary source and the documentation insufficient for assessment. Reliability score of 4 (not assignable).	
OECD 203	*Oryzias latipes* (Japanese medaka)	Dibenzoyl peroxide Purity: Information not available Test concentrations (mg/L): Information not available	LC_{50} (96 hr, static): 3.9 mg/L* *Unknown if the results are based on the nominal or mean measured concentration.	Supporting study. GLP compliant. Not enough information available to evaluate the test design, conditions, and validity. Reliability score of 4 (not assignable).	Anonymous, 1996. ECHA dissemination site, 2021.
Aquatic Invertebrates					
Method	**Species**	**Test material**	**Results**	**Remarks**	**Reference**
OECD 202; EU Method C.2	*Daphnia magna*	Dibenzoyl peroxide Purity: 74.6% Test concentrations (mg/L): Nominal: 0.427, 0.939, 2.07, 4.55 and 10. Mean measured: <LOQ, < LOQ, 0.0416, 0.0765 and 0.157.	EC_{50} (48 hr, static): 0.110 mg/L (mean measured)	Key study. GLP compliant. No deviations reported. Reliability score of 1 (reliable without restriction).	Anonymous, 2010b. ECHA dissemination site, 2021.
OECD (8.1) and EEC (8.2)	*Daphnia magna*	Dibenzoyl peroxide	EC_{50} (48 hr, static WAF): 2.91 mg/L	Supporting study.	Anonymous, 1999a.

guidelines		Purity: 74.4% Test concentrations (mg/L): Water Accommodated Fraction (WAF): negative control, undiluted WAF, 1:2, 1:4, 1:8 and 1:16 WAF dilutions.		GLP compliant. Deviations: test carried out as a WAF with series of dilutions. Insufficient information available to determine if the test substance concentration was maintained throughout the study. Reliability score of 3 (not reliable).	ECHA dissemination site, 2021.
OECD 202	*Daphnia magna*	Dibenzoyl peroxide Purity: 79.4% Test concentrations (mg/L): Nominal: 0.03, 0.06, 0.13, 0.25 and 0.5.	EC_{50} (48 hr, static): 0.07 mg/L (nom.)	Supporting study. GLP compliant. The concentration of dibenzoyl peroxide was less than 80% after 1 hr. Insufficient information available to evaluate the test design, conditions, and validity. Reliability score of 4 (not assignable).	Anonymous, 2001a. ECHA dissemination site, 2021.
Aquatic Algae					
Method	**Species**	**Test material**	**Results**	**Remarks**	**Reference**

OECD 201, EU Method C.3	*P.subcapitata*	Dibenzoyl peroxide Purity: 74.6% Test concentrations (mg/L): Nominal: 0.427, 0.939, 2.07, 4.55 and 10. Measured (initial): 0.034, 0.102, 0.166, 0.296, and 0.842 (benzoic acid expressed as dibenzoyl peroxide).	E_rC_{50} (72 hr, static): 0.0711 mg/L (ini. measured) E_bC_{50} (72 hr, static): 0.0422 mg/L (ini. measured) E_yC_{50} (72 hr, static): 0.0724 mg/L (ini. measured)	Key study. GLP compliant. No deviations reported. Reliability score of 1 (reliable without restriction).	Anonymous, 2010c. ECHA dissemination site, 2021.
OECD 9.1, EEC (9.2) and ISO Guidelines, (9.3) and ECETOC Monograph 26 (9.4)	*P.subcapitata*	Lucidol (Dibenzoyl peroxide) Purity: Information not available WAF: negative control, undiluted WAF, 1:2, 1:4, 1:8 and 1:16 and 1:32 WAF dilutions	E_rC_{50} (72 hr, static, WAF): 0.83 mg/L E_bC_{50} (72 hr, static, WAF): 0.44 mg/L	Supporting study. GLP compliant. Deviations: The $NaHCO_3$ concentration of the test medium was 150 mg/L instead of 50 mg/L, as recommended by the OECD/EEC Guidelines, in order to maintain a more constant pH during the test. It is stated the pH should not deviate more than 1.5 units during the test (EEC). The WAF method was used in the	Anonymous, 1999b. ECHA dissemination site, 2021.

				form of as a series of dilutions. Reliability score of 3 (not reliable).	
OECD 201	*P.subcapitata*	Dibenzoyl peroxide Purity: 79.4% Test concentration (mg/L) Nominal: 0.05, 0.1, 0.2, 0.4 and 0.8.	E_rC_{50} (72 hr, static): 0.44 mg/L. E_bC_{50} (72 hr, static): 0.07 mg/L	Supporting study. GLP status: not reported. Source of test species unknown. Insufficient information available to evaluate the test design, conditions, and validity. Reliability score of 4 (not assignable).	Anonymous, 2001b. ECHA dissemination site, 2021.

11.5.1 Acute (short-term) toxicity to fish

The acute toxicity of dibenzoyl peroxide to fish was assessed in four acute toxicity studies using different fish species and in accordance with international standards or accepted guidelines, equivalent to the OECD 203 guideline. Three of the four studies were GLP compliant.

The acute toxicity of dibenzoyl peroxide to *Oncorhynchus mykiss* (rainbow trout) was investigated under semi-static conditions (daily renewal) for 96 hours (Anonymous, 2010a). Following a preliminary range-finding study, juvenile rainbow trout were exposed for 96 hr to an aqueous solution of dibenzoyl peroxide at different nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg/L (geometric mean measured concentrations of 0.0081, 0.0097, 0.0224, 0.0316 and 0.0741 mg/L). All validity criteria, as set out in the OECD 203, were fulfilled. Hyperventilation was observed at the highest test concentration (10 mg/L nominal) in 1/7 fish after 72 hours and 2/7 fish after 96 hr. No mortalities were observed. The dossier submitter notes that the actual exposure concentrations of dibenzoyl peroxide were significantly lower than the nominal concentrations used in the test, especially at the higher test concentrations. This, in part, may have been influenced by the physicochemical properties of the substance such as the low water solubility (0.35 mg/L), high absorptive potential (log Koc 3.8) and the unstable nature of dibenzoyl peroxide in water. The study summary reports, based on the conditions of the study, the 96 hr (semi-static) LC_{50} and NOEC of

rainbow trout exposed to dibenzoyl peroxide to be 0.0602 mg/L and 0.0316 mg/L (mean measured), respectively.

The acute toxicity of dibenzoyl peroxide to *Poecilia reticulata* (Guppy) fish was investigated under aerated semi-static conditions for 96 hours at nominal concentrations of 0.7, 1.3, 2.4 and 4.2 mg/L following a dose range finding study (Anonymous, 1989). In the highest treatment group, 4.2 mg/L (nominal), 100% mortality was observed after 4 hr. In addition, 10% (1/10) and 70% (7/10) mortality was reported in the 1.3 and 2.4 mg/L (nominal) treatment groups after 48 hr, respectively. The study summary reports, based on the conditions of the study, the estimated 96 hr LC_{50} and NOEC of dibenzoyl peroxide for guppy fish, based on nominal concentrations, to be 2 mg/L (95% C.I. 1.7 and 2.4 mg/L) and 0.7 mg/L, respectively. The study summary indicates that the test species were obtained from a local aquarium retailer and therefore the provenance is uncertain and not in line with the recommendations of the test guideline. The life-stage of the test species is unknown, the length of the test organisms exceeded the recommendations of the test guideline (3 cm vs recommended length range of 1-2 cm, rationale not provided), and analytical monitoring was not performed. The study deviated from the recommendations of the OECD 203 guideline. The validity criteria as set out in the OECD 203 guideline were not fulfilled. Based on the above considerations, the dossier submitter agrees with the registrant's conclusion and considers that the study is not reliable. Refer to section 4.3.1 of Annex I to this CLP report for further study details.

The acute toxicity of dibenzoyl peroxide to *Oryzias latipes* (Japanese medaka) fish was investigated under continuous flow-through conditions for 96 hours (Anonymous, 2002). Fish were exposed to nominal concentrations of 0.25, 0.5, 1.0, 2.0 and 4 mg/L dibenzoyl peroxide (mean measured concentrations of 0.23, 0.47, 0.69, 1.54 and 2.17 mg/L). Precipitation of the test substance was observed at the surface of the test medium at 0.47 mg/L (mean measured) and 2.17 mg/L (mean measured) test concentrations. No mortality was observed in the controls or the 0.23 mg/L (mean measured) test group at the end of the study period. 100 % mortality was observed at 24 hr in the 1.54 and 2.17 (mean measured) mg/L test groups, at 48 hr for the 0.69 (mean measured) mg/L test group, and at 72 hr for the 0.47 (mean measured) mg/L test group. Under the conditions of the study, the 96 hr LC_{50} and NOEC of dibenzoyl peroxide for Japanese medaka fish, based on mean measured concentrations, were estimated to be 0.24 mg/L (95% C.I. 0.20 and 0.27 mg/L) and 0.23 mg/L, respectively. The study summary indicates that the source of the test species was unknown. The length of the test species was outside of the recommended range (3.5 cm vs recommended length range of 1-2 cm, rationale not provided). According to the literature, the body length of Japanese medaka at sexual maturity ranges from between 2.5-3 cm (Shiema et al., 2004). The dossier submitter agrees the identified points as reported in the study summary are deviations from the recommendations of the OECD 203 guideline. There is no further information reported in the study summary to assess against the validity criteria in accordance with the OECD 203 guideline. Based on the above considerations, the dossier submitter considers that the study is not reliable. Refer to section 4.3.1 of Annex I for further study details.

The acute toxicity of dibenzoyl peroxide to *Oryzias latipes* (Japanese medaka) fish was investigated in a non-GLP compliant study, under static conditions for 96 hours (Anonymous, 1996). Information on the test species, test concentrations, conditions, and design are not reported in the study summary. The study summary reports a 96 hr LC_{50} of 3.9 mg/L for Japanese medaka fish exposed to dibenzoyl peroxide. As no further information is reported in the study summary, the dossier submitter does not consider the study reliable.

In summary, the dossier submitter considers the 96 hr LC_{50} for *Oncorhynchus mykiss* (Anonymous, 2010a) (96 hr LC_{50} = 0.0602 mg/L (mean measured)) to be the lowest reliable value for this trophic level.

Data used for classification: Fish 96 hr LC_{50} = 0.0602 mg/L (mean measured)

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

The acute toxicity of dibenzoyl peroxide to aquatic invertebrates (*Daphnia magna*) was investigated in three acute toxicity studies and in accordance with international standards or accepted guidelines, equivalent to the OECD 202. All three studies were GLP compliant.

The acute toxicity (immobilisation) of dibenzoyl peroxide to *Daphnia magna* was investigated under static conditions for 48 hours (Anonymous, 2010b). Following a preliminary range finding study, neonate daphnids were exposed for 48 hr to an aqueous solution of dibenzoyl peroxide at nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg/L (mean measured: <LOQ, < LOQ, 0.0416, 0.0765 and 0.157 mg/L). Analytical monitoring was performed. Measured concentrations of dibenzoyl peroxide were significantly lower than the nominal concentrations used in the test, especially at the higher test concentrations. The concentration of dibenzoyl peroxide in the fresh samples was determined to be between 0 - 2% of the nominal concentrations. Dibenzoyl peroxide was not detectable in the expired samples (48 hr). Benzoic acid concentrations were detected in the fresh samples, but were undetectable in the expired solutions. The validity criteria as set out in OECD 202 were fulfilled. No mortalities were observed. The study summary reports that, under the conditions of the study, the estimated 48 hr EC_{50} and NOEC of dibenzoyl peroxide to *Daphnia magna* to be 0.110 mg/L (mean measured) (95% confidence limits of 0.0765 and 0.157 mg/L) and 0.0765 mg/L (mean measured), respectively.

The acute toxicity of dibenzoyl peroxide to *Daphnia magna* was investigated under static conditions using WAF serial dilutions for 48 hours (Anonymous, 1999a). Neonate daphnids were exposed to a negative control, undiluted WAF and 1:2, 1:4, 1:8 and 1:16 WAF dilutions of dibenzoyl peroxide solution. The WAF EC_{50} and NOEC (48 hr) of dibenzoyl peroxide to *Daphnia magna* were estimated to be 2.91 mg/L (95% confidence limits of 2.71-3.11 mg/L) and 1.99 mg/L, respectively. Although the validity criteria of the study appear to be fulfilled, the dossier submitter does not consider the study to be reliable for the following reasons: the WAF method was used in the form of a series of dilutions. ECHA's *Information Requirements Chapter R.7b: Endpoint Specific Guidance (2017)*, indicates that the WAF test method is generally used for

substances that contain many constituents or for any substance with very low water solubility. It also indicates that all efforts should first be made to produce a reliable and stable test concentration, and only if this is not feasible, due to the properties of the substance or due to disproportionate efforts, can the WAF be considered as a last resort to generate exposure in a test. The guidance also indicates that the method used to prepare the WAF should be fully described in the test report, with evidence provided of attainment of equilibrium and its compositional stability over time if possible. It also stated that WAFs are to be prepared individually and not by serial dilution of a single WAF stock. The dossier submitter notes that the WAF dilutions were prepared from serial dilutions and monitoring was performed at the beginning and end of the test only. No further information is reported in the study summary to indicate that the WAF method was the last resort. The reported effective test concentration was well above the water solubility of the substance. Finally, there is no further information reported in the robust study summary to determine if the test substance concentration was maintained throughout the study. Considering this, the dossier submitter does not consider the study to be reliable. Refer to section 4.3.2 of Annex I for further details on the study.

The acute toxicity of dibenzoyl peroxide to *Daphnia magna* was investigated under static conditions for 48 hours (Anonymous, 2001a). At 48 hr, 100% immobilisation was observed in the 0.13, 0.25 and 0.50 mg/L test groups while 10% immobilisation was observed in the 0.06 mg/L. The nominal 48 hr EC_{50} of dibenzoyl peroxide to *Daphnia magna* was estimated to be 0.07 mg/L. No further information is reported in the study summary on the test conditions, design, and results. The dossier submitter does not consider the study to be reliable.

In summary, the dossier submitter considers the 48 hr EC_{50} for *Daphnia magna* (Anonymous, 2010b) (48 hr EC_{50} = 0.110 mg/L (mean measured)) to be the lowest reliable value for this trophic level.

Data used for classification: aquatic invertebrates 48 hr EC_{50} = 0.110 mg/L (mean measured)

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

The acute toxicity of dibenzoyl peroxide to algae was investigated in three acute toxicity studies conducted in accordance with international standards or accepted guidelines, equivalent to OECD 201. Two of the three studies were GLP compliant.

The acute toxicity (growth inhibition) of dibenzoyl peroxide to *Pseudokirchneriella subcapitata* was investigated under static conditions for 72 hours (Anonymous, 2010c). Based on the results of a preliminary range finding study, algae were exposed to nominal test concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg/L (0.034, 0.102, 0.166, 0.296, and 0.842 mg/L measured as benzoic acid). The validity criteria, as set out in the OECD 201, were fulfilled. Measured concentrations of dibenzoyl peroxide were significantly lower than the nominal concentrations used in the test, especially at the higher test concentrations. The concentration of dibenzoyl peroxide in the fresh samples were determined to be between 0 - 2% of the nominal concentrations. Dibenzoyl peroxide was not detectable in the expired samples (72 hr). Benzoic acid

concentrations were detected in the fresh samples but were undetectable in the expired solutions except in the two highest test concentrations. The study summary reports that no microscopic abnormalities of the cells were detected. The study summary reports that, under the conditions of the study, the 72 hr E_bC_{50}, E_rC_{50}, E_yC_{50}, and NOEC of dibenzoyl peroxide to algae were 0.0422 mg/L, 0.0711 mg/L, 0.0724 mg/L and 0.02 mg/L respectively, based on initial measured concentrations in the fresh samples.

The acute toxicity of Lucidol (dibenzoyl peroxide) to *Pseudokirchneriella subcapitata* was investigated under static conditions using WAF serial dilutions for 72 hours (Anonymous, 1999b). Algae were exposed to a negative control, undiluted WAF, and 1:2, 1:4, 1:8, 1:16 and 1:32 WAF dilutions of Lucidol solution. Chemical analysis of the test concentration were performed using NPOC (non-purgeable organic carbon). Under the conditions of the study, the WAF E_bC_{50} and E_rC_{50} (72 hr) of dibenzoyl peroxide to algae (*Pseudokirchneriella subcapitata*), based on indicative concentrations, were estimated to be 0.44 mg/L (0.31-0.62 mg/L 95% confidence limits) and 0.83 mg/L (0.59-1.13 mg/L 95% confidence limits), respectively. The study summary reports that an indicative NOEC and LOEC of 0.12 mg/L and 0.23 mg/L were determined, respectively. Based on the information reported in the study summary the dossier submitter does not consider it possible to determine if the validity criteria of the study were fulfilled. In addition, the test concentrations were determined by NPOC analysis. The dossier submitter notes that NPOC analysis is not a specific method for the test compound and therefore the results can only be used as an indication of the concentration of the test material present. The WAF method was used in the form of a series of dilutions. ECHA's *Information Requirements Chapter R.7b: Endpoint Specific Guidance (2017)*, indicates that the WAF test method is generally used for substances that contain many constituents or for any substance with very low water solubility. It also indicates that all efforts should first be made to produce a reliable and stable test concentration, and only if this is not feasible, due to the properties of the substance or due to disproportionate efforts, can the WAF be considered as a last resort to generate exposure in a test. No further information is reported in the study summary to indicate that the WAF method was the last resort. Based on the above considerations, the dossier submitter does not consider the study reliable. Refer to section 4.3.3 of Annex I for further details on the study.

In a final acute toxicity study on algae, *Pseudokirchneriella subcapitata*, were exposed to nominal concentrations of dibenzoyl peroxide (0.05, 0.1, 0.2, 0.4 and 0.8 mg/L) under static conditions for 72 hours (Anonymous, 2001b). Under the conditions of the study the nominal 72 hr E_bC_{50} and E_rC_{50} of dibenzoyl peroxide to algae, were estimated to be 0.07 mg/L and 0.44 mg/L, respectively. The dossier submitter considers, based on the information reported in the study summary on the test design, conditions, results and validity, that the study is not reliable. Please refer to section 4.3.3 of Annex I for further details on the study.

In summary, the dossier submitter considers the 72 hr E_rC_{50} for *Pseudokirchneriella subcapitata* (Anonymous, 2010c) (72 hr E_rC_{50} = 0.0711 mg/L (ini. measured)) to be the lowest reliable value for this trophic level.

Data used for classification: algae 72 hr E_rC_{50} = 0.0711 mg/L (ini. measured).

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No relevant data available.

11.6 Long-term aquatic hazard

Table 12: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
No data available.					
Aquatic Invertebrates					
OECD 211	*Daphnia magna*	Dibenzoyl peroxide Purity: 74.2% Test conc.: Nominal: 3.2, 5.6, 10, 18 and 32% v/v saturated solution. Time-weighted average (TWA) mean measured: 0.00062, 0.0011, 0.0016, 0.0028 and 0.0074 mg/L.	EC_{10} (reproduction): 0.001 mg/L (95%C.I. 0.00010-0.0018) (semi-static, TWA mean measured). NOEC (reproduction): 0.0011 mg/L (semi-static, TWA mean measured). 50% (statistically significant) mortality (immobilization) at 0.0028 and 0.0074 mg/L (mean measured). Statistically significant reduction in no. live offspring/adult in test groups 0.0016, 0.0028 and 0.0074 mg/L. Observations: a number of parent daphnia reported as pale at all test conc. compared to the control.	Key study. GLP compliant. No deviations reported. Reliability score of 1 (reliable without restriction).	Anonymous, 2015a. ECHA dissemination site, 2021.
Aquatic Algae					
Method	**Species**	**Test material**	**Results**	**Remarks**	**Reference**
OECD 201, EU Method C.3	*P.subcapitata*	Dibenzoyl peroxide Purity: 74.6% Test	NOEC (72 hr, static): 0.02 mg/L (ini. measured).	Key study. GLP compliant. No deviations	Anonymous, 2010c. ECHA dissemination

Method	Species	Test material	Results	Remarks	Reference
		concentrations (mg/L): Nominal: 0.427, 0.939, 2.07, 4.55 and 10. Measured (initial): 0.034, 0.102, 0.166, 0.296, and 0.842 (benzoic acid expressed as dibenzoyl peroxide).		reported. Reliability score of 1 (reliable without restriction).	site, 2021.
OECD 9.1, EEC (9.2) and ISO Guidelines, (9.3) and ECETOC Monograph 26 (9.4)	*P.subcapitata*	Lucidol (Dibenzoyl peroxide) Purity: information not available WAF: negative control, undiluted WAF, 1:2, 1:4, 1:8 and 1:16 and 1:32 WAF dilutions	NOEC (72 hr, static, WAF): 0.12 mg/L. LOEC (72 hr, static, WAF): 0.23 mg/L.	Supporting study. GLP compliant. Deviations: The $NaHCO_3$ concentration of the test medium was 150 mg/L instead of 50 mg/L, as recommended by the OECD/EEC Guidelines, in order to maintain a more constant pH during the test. It is stated the pH should not deviate more than 1.5 units during the test (EEC). The WAF method was used in the form of as a series of dilutions.	Anonymous, 1999b. ECHA dissemination site, 2021.

Method	Species	Test material	Results	Remarks	Reference
				Reliability score of 3 (not reliable).	

11.6.1 Chronic toxicity to fish

No relevant data available.

11.6.2 Chronic toxicity to aquatic invertebrates

One long-term freshwater toxicity test on aquatic invertebrates is available (Anonymous, 2015a), investigating the effects of dibenzoyl peroxide on the reproductive output of *Daphnia magna* according to OECD 211. Daphnids (< 24 hr old at the start of the test, 10 individuals per treatments, held individually) were exposed to dibenzoyl peroxide under semi-static conditions for 21 days. The saturated solution method was used to prepare the test solution. A nominal amount of test material (20 mg) was dispersed in 2 litres of test water with the aid of sonication for 30 minutes. Undissolved test substance was removed by filtration through a 0.2 µm Gelman Acrocap filter to give a 100% v/v saturated solution. A series of dilutions were made from this saturated solution to give the required test concentrations of 3.2, 5.6, 10, 18 and 32% v/v (nominal) saturated solution, corresponding to time-weighted mean measured test concentrations of 0.00062, 0.0011, 0.0016, 0.0028 and 0.0074 mg/L. The test solution was renewed daily. The control group was maintained under identical conditions with the exception of being exposed to the test substance. The concentration and stability of the test substance in the test preparations were verified by chemical analysis on days 0, 1, 6, 7, 13, 14, 20 and 21. Water quality measurements and temperature were monitored throughout the test. Daily observations were made on the number of dead/surviving adult daphnids, dead/surviving offspring, and the number of discarded unhatched eggs. The general condition and size of the parental daphnia was assessed and compared with the controls. The number of daphnia with eggs or young in the brood pouch was determined daily. At the end of the test, the length of each surviving parent animal was determined. The percentage parental survival and total number of live young exposed to dibenzoyl peroxide for 21 days are reported in Table 13.

Table 13: Parental survival and total number of live young following exposure of *Daphnia magna* to dibenzoyl peroxide for 21 days (Anonymous, 2015a, ECHA Dissemination site, 2021)

Nominal (%v/v saturated solution)	Mean Measured (TWA mg/L)	P1 Generation % Survival (mortality)	Total No. Live Young	Total No. Live Young ex. Replicates with Parental Accidental or Inadvertent Mortalities*	No. Live Young/Parent at start of test ex. Replicates with Parental Accidental or Inadvertent Mortalities*
Control	Control	100 (0)	1083	1046	116

3.2	0.00062	80 (20)	924	892	112
5.6	0.0011	89 (11)	1056	900	113
10	0.0016	90 (10)	862	862	96**
18	0.0028	50 (50)	593	593	59**
32	0.0074	50 (50)	363	363	36**

* Excluding Replicates with Parental Accidental and/or Inadvertent Mortalities

** Statistically significant difference (reduction) in the number of live offspring per adult compared to the control.

Significant mortality (immobilization) was observed at test concentrations of 0.0028 and 0.0074 mg/L (mean measured) resulting in 50% mortality in both test groups by Day 21 indicating a prolonged toxic effect following exposure of *Daphnia magna* to dibenzoyl peroxide. Lower levels of immobilisation, between 10 and 20%, were observed at the test concentrations of 0.00062, 0.0011 and 0.0016 mg/L (mean measured). Throughout the test, some of the parent daphnia, in all test concentrations, were observed as pale when compared to the control daphnia. The robust study summary reports that there were no statistically significant differences (P 0.05) between the control and each test group in terms of length of the daphnids after 21 days exposure to the test substance. The results of the time to first brood, the time to production of first brood, and the average body lengths of the 1st generation surviving adults were not reported. After 21 days there were no statistically significant differences in the number of live offspring produced per adult between the control and the 0.00062 and 0.0011 mg/L test groups. There was a statistically significant reduction in the number of live offspring per adult in the 0.0016, 0.0028 and 0.0074 mg/L test groups (mean measured) when compared to the control after 21 days. The total number of live offspring per adult recorded for the 0.0016, 0.0028 and 0.0074 mg/L test groups were 96, 59 and 36 respectively while the control group recorded 116 live offspring per adult.

The validity criteria, as set out in the OECD 211, were fulfilled. The mortality in the control group of adult *Daphnia magna* was 0%; the mean number of offspring produced per control adult was 116; and the coefficient of variation around the mean number of offspring produced per control adult was 13.5%.

Under the conditions of the study, an EC_{10} (reproduction) of 0.001 mg/L (95% C.I. 0.00010-0.0018), based on the time-weighted mean measured test concentrations, was determined for *Daphnia magna* exposed to dibenzoyl peroxide for 21 days. A NOEC (reproduction) of 0.0011 mg/L (TWA mean measured) was also derived based on the statistically significant differences (reduction) in the number of live offspring per adult compared to the control after 21 days.

Data used for classification: invertebrates EC_{10} = 0.001 mg/L (TWA mean measured)

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No relevant data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Table 14: Comparison with criteria for acute aquatic hazards

	Criteria for acute environmental hazards	Dibenzoyl peroxide	Conclusion
Acute Aquatic Toxicity	Category 1: $LC_{50}/EC_{50}/E_rC_{50} \leq 1$ mg/L	Fish: 96 hr LC_{50} = 0.0602 mg/L (mean measured) (*Oncorhynchus mykiss)* Invertebrates: 48 hr LC_{50}= 0.110 mg/L (mean measured) (*Daphnia magna*) Algae: 72 hr E_rC_{50}= 0.0711 mg/L (ini. measured) (*P.subcapitata*)	**Aquatic Acute 1,** **M factor=10**

Acute toxicity studies are available for all three trophic levels. The results presented in Table 14 above and discussed in more detail in sections 11.5.1 to 11.5.3, demonstrate that dibenzoyl peroxide is acutely toxic to fish, invertebrates and algae with all endpoints below 1 mg/L (< 1mg/L) for all three species.

The CLP Regulation sets a criteria value of < 1 mg/L for hazard category 1 acute toxicity. In accordance with the CLP Regulation, the most protective and valid short-term toxicity endpoint (LC_{50} or EC_{50}) should be compared to the acute toxicity criteria and used for classification purposes. The dossier submitter considers the 96 hr LC_{50} of 0.0602 mg/L (mean measured) for fish, *Oncorhynchus mykiss*, to be the lowest reliable acute effect concentration. Dibenzoyl peroxide fulfils the criteria for classification as Category 1 Aquatic Acute; H400 '*Very toxic to aquatic life'* according to the CLP Regulation. In accordance with Annex I: Table 4.1.3 of the CLP Regulation, for mixture toxicity, a corresponding M factor of 10 is applicable for an acute toxicity endpoint between 0.01 and 0.1 mg/L.

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

Table 15: Comparison with criteria for long-term aquatic hazards

	Criteria for acute environmental hazards	Dibenzoyl peroxide	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)	Hydrolytically unstable Half-life < 24 hr 68 % ThOD after 28 days = readily biodegradable	**Rapidly degradable**
Bioaccumulation	log Kow ≥ 4 BCF ≥ 500	log Kow 3.43 (estimated) log Kow = 3.2 (measured) BCF = 89.11 L/kg (estimated)	**Low potential for bioaccumulation**
Aquatic toxicity	*Chronic toxicity:* Rapidly degradable substances: Cat. 1: EC_x or NOEC ≤ 0.01 mg/L Cat. 2: EC_x or NOEC ≤ 0.1 mg/L Cat. 3: EC_x or NOEC ≤ 1 mg/L	Invertebrates: 21 day EC_{10} = 0.001 mg/L (*Daphnia magna*) Algae: 72 hr NOEC = 0.02 mg/L (static) (*P. subcapitata*)	**Aquatic Chronic 1,** **M factor=10** *(based on invertebrate EC_{10})*

Dibenzoyl peroxide is considered rapidly degradable based on the results from the available ready biodegradability studies (68% degradation after 28 days) and hydrolysis study (half-life < 24 hr; rapidly hydrolyses). There is no available experimental information on the bioaccumulation of dibenzoyl peroxide. The measured (OECD 117 octanol-water partition coefficient study) and estimated (predictive modelling) log Kow values are below the cut-off criteria of 4. Dibenzoyl peroxide has an estimated BCF value of < 100 which is less than the CLP BCF criteria of ≥ 500. Therefore, dibenzoyl peroxide can be considered to have a low bioaccumulation potential for classification purposes. The dossier submitter notes that benzoic acid (EC No. 200-618-2) is considered the main degradation product of dibenzoyl peroxide. The available data for benzoic acid, as reported in the REACH registration dossier, indicates that benzoic acid can be considered as readily biodegradable and has a low bioaccumulation potential (ECHA dissemination site, 2021b).

One valid long-term toxicity study is available for aquatic invertebrates, a 21 day semi-static freshwater reproduction study was performed with *Daphnia magna*. A 72 hr freshwater static algae study, reporting an NOEC of 0.02 mg/L (ini. measured), is also available (Anonymous, 2010c). Based on the available long-

term data and the physical-chemical properties of dibenzoyl peroxide (rapidly degradable; BCF < 500; log Kow < 4) the use of the surrogate approach for chronic classification is not considered appropriate.

The CLP Regulation sets a criteria value of ≤ 0.01 mg/L (rapidly degradable substances) for hazard category 1 chronic toxicity. In accordance with the CLP Regulation, the most protective and valid long-term toxicity endpoint (EC_x or NOEC) should be compared to the chronic toxicity criteria and used for classification purposes. The 21 day EC_{10} of 0.001 mg/L dibenzoyl peroxide (mean measured) for invertebrates, *Daphnia magna*, is the lowest effect concentration. Dibenzoyl peroxide fulfils the criteria for classification as Category 1 Aquatic Chronic; H410 '*Very toxic to aquatic life with long lasting effects'* according to CLP Regulation for a rapidly degradable substance. For mixture toxicity, a corresponding M factor of 10 is applicable for a chronic toxicity endpoint between < 0.0001 and ≤ 0.001 mg/L (rapidly degradable substances).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute Aquatic Hazards:

Dibenzoyl peroxide is acutely toxic to fish, invertebrates and algae. In accordance with the CLP Regulation, the most protective and valid short-term toxicity endpoint for classification purposes (i.e. LC_{50} or EC_{50}) is the 96 hr LC_{50} of 0.0602 mg/L (mean measured) for fish, *Oncorhynchus mykiss*. Dibenzoyl peroxide fulfils the criteria for the classification as Category 1 Aquatic Acute; H400 '*Very toxic to aquatic life'* according to CLP Regulation. The corresponding M factor for an acute endpoint between 0.01 and 0.1 mg/L to be considered for mixture toxicity is 10 (M=10).

Chronic Aquatic Hazards:

Dibenzoyl peroxide is rapidly degradable and has a low potential to bioaccumulate. Dibenzoyl peroxide is chronically toxic to invertebrates, with the most protective valid long-term toxicity EC_{10} and NOEC of 0.001 mg/L (mean measured) and 0.0011 mg/L (mean measured), respectively. Dibenzoyl peroxide fulfils the criteria for the classification as Category 1 Aquatic Chronic; H410 '*Very toxic to aquatic life with long lasting effects'* according to CLP Regulation for a rapidly degradable substance. The corresponding M factor for a chronic endpoint between < 0.0001 and ≤ 0.001 mg/L for a rapidly degradable substance to be considered for mixture toxicity is 10 (M=10).

RAC evaluation of aquatic hazards (acute and chronic)
Summary of the Dossier Submitter's proposal

The current entry in Annex VI of Regulation (EC) No 1272/2008 of dibenzoyl peroxide does not include harmonised classification for environmental hazards.

The Dossier Submitter (DS) proposed to classify the substance as:

- **Aquatic Acute 1 (H400) with M-factor of 10** based on a 96 h EC_{50} value of 0.0602 mg/L for fish *Oncorhynchus mykiss*.
- **Aquatic Chronic 1 (H410) with M-factor of 10** based on 21 d EC_{10} value of 0.001 mg/L for the invertebrate *Daphnia magna.* The substance has low bioaccumulation potential and is rapidly degradable.

Rapid degradability

Hydrolysis

Two hydrolysis studies according to OECD TG 111 are available. In the first study, dibenzoyl peroxide was determined to be hydrolytically unstable at acidic (pH 4), neutral (pH 7) and alkaline (pH 9) conditions at 50 °C. The rate of hydrolysis increased with increasing pH and at pH 9 resulted in the availability of approximately 20 % of the applied test substance. Greater than 50 % hydrolysis occurred after 2.4 hours, equivalent to a half-life (DT_{50}) of less than 1 day under environmentally relevant condition (25 °C). The principal hydrolysis product, benzoic acid, was detected. Benzoic acid can be considered as readily biodegradable and has a low bioaccumulation potential (log K_{OW} < 2) (REACH registration dossier, ECHA, 2021b). Benzoic acid does not have a harmonised classification entry for environmental hazards. In the second study, dibenzoyl peroxide degraded 93.5, 94.1 and 94.2 % at pH 4, 7 and 9 respectively by day 5 and at 50 ºC. The half-life of dibenzoyl peroxide at pH 4 and 7 was determined to be 11.9 and 5.2 hours at 25 °C, respectively, while the half-life at pH 9 and 25 °C could not be determined as dibenzoyl peroxide was not detected. The study is considered not reliable as not enough data is available to verify the validity of the study or the observed results.

Ready biodegradability

There are four ready biodegradability studies with conflicting results and with different reliability available on dibenzoyl peroxide. In the first study (Reliability 1) (Anonymous, 2015b) the biodegradation of dibenzoyl peroxide was determined with ready biodegradability closed bottle test (OECD TG 301 D). Secondary activated sludge (non-adapted, 0.4 g (DW)/L pre-conditioned) obtained from the Nieuwgraaf wastewater treatment plant in Duiven (Netherlands) was exposed to 2 mg/L dibenzoyl peroxide over 28 days at 22-24 °C. Under the test conditions, dibenzoyl peroxide reported a theoretical oxygen demand (ThOD) of 2.7 mg/L, corresponding to 71 % degradation after 28 days. The validity criteria of the test were fulfilled. Dibenzoyl peroxide can be considered readily biodegradable under test conditions of the study.

In the second OECD TG 301 D test (Reliability 2) (Anonymous, 2009c), activated sludge (concentration equivalent to a maximum of 30 mg/L, pre-conditioned, non-adapted), prepared in the laboratory from secondary effluent from a wastewater treatment plant with activated sludge treating domestic wastewater in the municipality of Abidos (France) was exposed to 4 mg/L dibenzoyl peroxide for 28 days. The oxygen depletion in the inoculum blank did not exceed 1.5 mg/L after 28 days. However, the residual oxygen concentration remained above 0.5 mg/L. The reference substance, sodium benzoate, reached 42 % degradation by Day 14 which is below the validity threshold of ≥ 60 %. The validity criteria of the test were not

fulfilled. Under the conditions of the study, dibenzoyl peroxide biodegraded 68 % by Day 28, exceeding the 60 % threshold for this test system. However, this level of biodegradation was not achieved within the 10-day window. Dibenzoyl peroxide demonstrated inherent biodegradability under the conditions of the study.

In a further compliant OECD TG 301 D test (Reliability 2) (Anonymous, 1990), the ready biodegradability of dibenzoyl peroxide, 1.5 mg/L (applied using silica gel), was investigated in secondary activated sludge (non-adapted, pre-conditioned) from an activated sludge plant predominantly treating domestic wastewater in Duiven, Netherlands. After 28 days, dibenzoyl peroxide reportedly degraded by 56 %. The test was extended to 84 days; however, the level of degradation did not exceed 56 %. The inhibitory effects of dibenzoyl peroxide on the micro-organisms of the inoculum were not observed. Considering the extent of degradation, the DS considers that dibenzoyl peroxide did not fulfil the ready biodegradability criteria. Under the conditions of the study, it was concluded that dibenzoyl peroxide can be considered as not readily biodegradable. The DS noted that although the validity criteria of the OECD TG 301 guideline were fulfilled the following deviations were noted: secondary activated sludge was used as inoculum instead of recommended secondary effluent (or surface water). The OECD 301 D guideline recommends for substances with water solubility below 1 g/L that stock solutions are prepared in mineral medium or added directly to the mineral medium rather than in water/solvent as was performed in the study. A test concentration of 1.5 mg/L dibenzoyl peroxide was used instead of recommended test concentration of between 2 and 10 mg/L. It is unclear if these deviations affected the biodegradation potential of the test material or validity of the study.

The ready biodegradability of dibenzoyl peroxide was evaluated in accordance with an OECD Ready Biodegradability modified MITI test (OECD TG 301 C) for 21 days (Anonymous, 1992). Under the conditions of the study, dibenzoyl peroxide reported 88 % degradation after 21 days. The robust study summary concluded that dibenzoyl peroxide was readily biodegradable; however, no further information is reported in the study summary to verify the validity of the study. The study is not considered reliable (Reliability 4) by the DS.

The DS noted that benzoic acid (EC 200-618-2) was considered the main degradation product of dibenzoyl peroxide. The robust study summary for benzoic acid (REACH registration dossier) reports that benzoic acid can be considered as readily biodegradable (ECHA dissemination site, 2021b).

Conclusion on rapid degradability

Overall, the DS concluded that dibenzoyl peroxide is considered to be rapidly degradable in the environment.

Bioaccumulation

For dibenzoyl peroxide, the EPISuite KOWWIN (v1.68) and BCFBAF QSAR models predicted log K_{ow} and BCF values for dibenzoyl peroxide of 3.43 and 89.11 L/kg, respectively.

Measured octanol-water partition coefficient (log K_{OW}) determined according to OECD TG 117 was 3.2 at 22 °C.

Aquatic bioaccumulation studies to determine the bioconcentration of dibenzoyl peroxide in aquatic species were not available.

The DS concluded that dibenzoyl peroxide has a low potential for bioaccumulation.

Aquatic Toxicity

The summary of the relevant information on aquatic toxicity for dibenzoyl peroxide are provided in the following table (the key endpoints used in hazard classification are highlighted in bold). Studies that were considered unreliable by the DS were not provided in the Table.

Table: *Summary of relevant information on aquatic toxicity for dibenzoyl peroxide*

Method/Exposure	Test organism	Endpoint	Toxicity values (mg/L)	Reference/Remarks
Acute aquatic toxicity				
OECD 203; EU Method C.1, GLP Semi-static Purity: 74.6 %	*Oncorhynchus mykiss*	96 h LC_{50}	**0.0602 im***	Anonymous, 2010a. ECHA dissemination site, 2021
OECD 202; EU Method C.2, GLP Static Purity: 74.6 %	*Daphnia magna*	48 h EC_{50}	0.11 im*	Anonymous, 2010b. ECHA dissemination site, 2021
OECD 201, EU Method C.3, GLP Static Purity: 74.6 %	*Pseudokirchneriella subcapitata*	72 h E_rC_{50} 72 h E_bC_{50} 72 h E_yC_{50}	0.0711 im 0.0422 im 0.0724 im	Anonymous, 2010c. ECHA dissemination site, 2021
Long-term toxicity				
OECD 211, GLP Semi-static Purity: 74.2 %	*Daphnia magna*	21 d EC_{10} (reproduction) 21 d NOEC (reproduction)	0.001 TWA mm 0.0011 TWA mm	Anonymous, 2015a. ECHA dissemination site, 2021
OECD 201, EU Method C.3, GLP Static Purity: 74.6 %	*Pseudokirchneriella subcapitata*	72 h NOE_rC	0.02 im	Anonymous, 2010c. ECHA dissemination site, 2021

Notes:
mm - mean measured concentration
im - initial measured concentration
TWA mm - Time-weighted average (TWA) measured concentration
*** In the CLH report the endpoints are based on mean measured concentrations but based on comment (No 3) received in public consultation this was changed to initial measured concentrations.**

Acute aquatic toxicity data on dibenzoyl peroxide are available for all three trophic levels (fish, invertebrates and algae). One study per trophic level was evaluated in the CLH report as reliable for classification purposes. The most sensitive acute toxicity value for fish is 96 h LC_{50} of 0.0602 mg/L for *Oncorhynchus mykiss,* for invertebrates is 48 h EC_{50} of 0.11 mg/L for *Daphnia magna* and for algae is 72 h E_rC_{50} of 0.0711 mg/L for *Pseudokirchneriella subcapitata.* All aquatic acute toxicity values were below the threshold value of 1 mg/L. The acute aquatic classification proposed by the DS was based on the toxicity value for fish (*Oncorhynchus mykiss*, 96 h LC_{50} of 0.0602 mg/L) which is the most acutely sensitive taxonomic group. The DS proposed **Aquatic Acute 1** (H400) with an acute **M-factor of 10** ($0.01 < L(E)C_{50} \leq 0.1$ mg/L).

Chronic aquatic toxicity data on dibenzoyl peroxide is available for invertebrates and algae. One invertebrate and one algae study were evaluated in the CLH report as reliable for

classification purposes. The most sensitive chronic endpoint for invertebrates is 21 d EC_{10} value of 0.001 mg/L and 21 d NOEC value of 0.0011 mg/L for *Daphnia magna* and for algae is 72 h NOE_rC value of 0.02 mg/L for *Pseudokirchneriella subcapitata.* The chronic aquatic classification proposed by the DS was based on the toxicity value for invertebrate (*D. magna*, 21 d EC_{10} = 0.001 mg/L) along with the understanding that the substance is rapidly degradable. The DS proposed **Aquatic Chronic 1** (H410) with a chronic **M-factor of 10** ($0.0001 < EC_{10} \leq 0.001$ mg/L). Based on the available long-term data and the physical-chemical properties of dibenzoyl peroxide (rapidly degradable; BCF < 500; log K_{OW} < 4) the use of the surrogate approach for chronic classification cannot be applied.

Comments received during consultation

Two Member States (MS), one individual, National Authority and company-importer provided comments.

The MSs agreed with the proposed classification for environmental hazards by DS. One MS provided detailed explanation of agreement with DS proposal.

An individual provided safety data sheet. The DS indicated that no additional hazard data were provided in the safety data sheet and the results of the reported studies in the safety data sheet were reflected in the CLH proposal.

A National Authority asked for clarification whether endpoints from acute toxicity studies with *Oncorhynchus mykiss* (Anonymous, 2010a) and *Daphnia magna* (Anonymous, 2010b) are based on initial measured or mean measured concentrations over the test period. In the CLH report the endpoints were based on mean measured concentrations while in EU REACH registration were based on initial measured concentrations. In addition, the National Authority asked RAC to consider which basis (initial measured or mean measured) is most relevant for the acute endpoints, noting the rapid hydrolysis of the test substance. The DS clarified that reported results are based on initial measured concentrations because in expired samples (at 24 h for fish and 48 h for daphnia) no measurable levels of dibenzoyl peroxide were found at any exposure concentration. DS pointed out that the same applies to acute study with *Pseudokirchneriella subcapitata* (Anonymous, 2010c).

The National Authority indicated that the key chronic endpoint for the proposed classification was *Daphnia magna* EC_{10} of 0.001 mg/L (95 % C.I. 0.00010-0.0018 mg/L) based on reproduction. Whilst within the test guideline recommendation, the National Authority noted that the coefficient of variation (CV) around the EC_{10} endpoint for mean number of living offspring produced per parent in the controls was 13.5 %. As this control CV was above 10 % and given the EC_{10} was below the NOEC (albeit it only slightly), the EC_{10} was likely to reflect considerable uncertainty regarding where a 10 % difference compared to the mean living offspring truly lies – this was also demonstrated by the confidence intervals. The National Authority asked for more information about the individual 10 control replicates to understand the background variation and the dose-response regression, e.g., if there was any outlier. The National Authority wondered if the reproduction NOEC of 0.0011 mg/L from the same study, or an EC_{20}, was more reliable and relevant to hazard classification in this instance. The NOEC value would lead to Aquatic Chronic 1 with M-factor of 1 for rapidly degradable substances. The DS indicated that in line with ECHA guidance R.10 (2017) EC_{10} is preferred over NOEC. Therefore, EC_{10} was considered more appropriate to derive the classification and M factor. The DS agreed with the National Authority that EC_{10} and NOEC lead to a classification of the

substance as Aquatic Chronic 1 but with different M-factors. The DS pointed out that in the robust study summary reported in the registration dossier no information was provided on the individual control replicates or on the dose-response regression and therefore it was not possible to provide any further information, including if there was any outlier in this group. The DS noted that the 95 % CI of the EC_{10} spans across three M factor levels, with only the current proposed M factor of 10 fully covered. The DS noted the difference in the number of decimal places for each value: 0.001 mg/L and 0.0011 mg/L for the EC_{10} and NOEC, respectively. No information is provided in the study summary as to whether the EC_{10} value was rounded up.

The company-importer pointed out that they do not have any toxicological and ecotoxicological studies to improve the proposal to give a support to the higher classification.

Assessment and comparison with the classification criteria

Degradation

The substance is hydrolytically unstable at pH 4-9 and 50 °C. Hydrolysis DT_{50} values are less than 1 day under environmentally relevant condition (25 °C and pH 4-9). The main degradation product was benzoic acid which has not been classified for environmental hazard but can be considered as readily biodegradable and has a low bioaccumulation potential. RAC notes that all reported L(E)C50 and NOEC/EC_{10} for fish, invertebrates and algae available on ECHA dissemination site (registered substances) are above the CLP criterion (acute and chronic) of 1 mg/L for acute and chronic hazard classification for rapidly degradable substance (data for ready biodegradability only are available).

There are three ready biodegradability studies (OECD TG 301D) with different reliability (Reliability 1 or 2) and conflicting results available:

- The study by Anonymous, 2015b (Reliability 1) showing a degradation of 71 % in 28 days. The study is considered the most reliable and best documented of all three (guidance criteria fulfilled, non-adapted inoculum and 10-day window criterion fulfilled).
- The study by Anonymous, 2009c (Reliability 2) did not reach the pass-level for readily biodegradability (10-day window criterion not fulfilled), but there is at least a substantial degradation (68 % in 28 days), which also contributes to the conclusion that the substance will not be persistent.
- The study by Anonymous, 1990 (Reliability 2) showed 56 % degradation after 28 days. This study was considered less reliable due to deviation from test guideline.

In line with the current CLP Guidance (Version 5.0, July 2017) "*positive results in ready biodegradability tests could be considered valid, irrespective of negative results, when the scientific quality is good and the test conditions are well documented, i.e., guideline criteria are fulfilled, including the use of non-pre-exposed (non-adapted) inoculum*". Consequently, RAC is of the opinion that dibenzoyl peroxide should be considered readily biodegradable following the study by Anonymous, 2015b.

RAC agrees with the DS proposal to consider dibenzoyl peroxide as rapidly degradable.

Bioaccumulation

The experimental log K_{OW} of 3.2 and estimated log K_{OW} value of 3.43 are below the CLP trigger value of Log K_{OW} ≥ 4. The low bioaccumulation potential of dibenzoyl peroxide is also supported by the estimated BCF value of 89.11 L/kg which is below the CLP criterion of BCF ≥ 500. Therefore, RAC agrees with the DS proposal to consider dibenzoyl peroxide as a substance with a low potential to bioaccumulate.

Acute toxicity

Reliable aquatic acute toxicity data on dibenzoyl peroxide are available for fish, invertebrates and algae. RAC notes that all acute toxicity endpoints ($L(E)C_{50}$) for fish, invertebrates and algae are below the threshold value of 1 mg/L. Fish are the most acutely sensitive group and the lowest toxicity value is the initial mean measured 96 h EC50 value of 0.0602 mg/L for rainbow trout *Oncorhynchus mykiss.* According to Table 4.1.0 (a) and 4.1.3 of the CLP guidance, dibenzoyl peroxide should be classified as Aquatic Acute 1 with an M-factor of 10.

Chronic toxicity

Reliable aquatic chronic toxicity data on dibenzoyl peroxide are available for two trophic levels, invertebrates and algae. Data for fish are lacking.

The lowest chronic effect value is obtained from a test with crustacea *Daphnia magna* and corresponds to the time-weighted mean measured 21 d EC_{10} of 0.001 mg/L. As this value is below the threshold value of 1 mg/L, the substance is considered rapidly degradable and the substance has low potential for bioaccumulation, RAC concludes that a classification as Aquatic Chronic 1 (H410) is justified. As $0.0001 < EC_{10} \leq 0.001$ mg/L, the chronic M-factor is 10.

In line with the CLP guidance section 4.1.3.3 and Table 4.1.0. the surrogate method is not applicable for dibenzoyl peroxide as the substance is considered rapidly degradable and does not fulfil the criteria for bioaccumulation.

In summary, based on the available data, RAC considers that dibenzoyl peroxide should be classified according to CLP as:

Aquatic Acute 1 (H400), M-factor = 10 and

Aquatic Chronic 1 (H410), M-factor = 10

This is consistent with the conclusion of the DS.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated as part of this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

- ECHA (2021a): Dibenzoyl peroxide registration dossier (accessed August 2021) https://echa.europa.eu/registration-dossier/-/registered-dossier/15164
- ECHA (2021b): Benzoic acid registration dossier (accessed August 2021) https://echa.europa.eu/registration-dossier/-/registered-dossier/13124
- Anonymous (1989): Acute Toxicity of Dibenzoyl peroxide to fish. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1999a): Acute Toxicity of Lucidol to Daphnia magna. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1990): Biodegradability of Dibenzoyl peroxide. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2015b): Biodegradability of Dibenzoyl peroxide (CAS No. 94-36-0) in the Closed Bottle Test (OECD 301D). (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1992): Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010d): Dibenzoyl peroxide Abiotic Degradation: Hydrolysis as a Function of pH (Preliminary Test) (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010b): Dibenzoyl peroxide: Acute Toxicity to *Daphnia magna*. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010a): Dibenzoyl peroxide: Acute Toxicity to Fish. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010c): Dibenzoyl peroxide Algal Growth Inhibition Assay. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2015a): Dibenzoyl peroxide (CAS No. 94-36-0): *Daphnia magna* Reproduction test. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2009a): Dibenzoyl peroxide – Adsorption Coefficient (Koc) on Soil and Sewage Sludge. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2009b): Dibenzoyl peroxide – Partition Coefficient (n-octanol/water) HPLC Method. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2009c): Dibenzoyl peroxide – Ready Biodegradability – Closed Bottle Test. (Unpublished report). ECHA Dissemination site 2021.

- Anonymous (1999b): Effects of the Water Accommodated Fraction of Lucidol on the Growth of the Freshwater Green Alga *Pseudokirchneriella subcapitata*. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1996): Establishment of Advanced Testing Methods for Hazardous Chemicals. ECHA Dissemination site 2021.
- Anonymous (2001a): The Acute Toxicity of Benzoyl peroxide to Aquatic Invertebrates (*Daphnia*). ECHA Dissemination site 2021.
- Anonymous (2002): The Acute Toxicity of Benzoyl peroxide to Fish. ECHA Dissemination site 2021.
- Anonymous (2001c): The Test of Benzoyl peroxide Hydrolysis as a Function of pH. ECHA Dissemination site 2021.
- Anonymous (2001b): The Toxicity of Benzoyl peroxide to Aquatic plants (algae). ECHA Dissemination site 2021.
- ECHA (2017): Guidance on the application of the CLP criteria.
- Klimisch H.J., Andreae M., and Tillmann U. (1997): A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25 (1), 1-5. DOI: 10.1006/rtph.1996.1076.
- Shima, A. and Mitani, H. (2004). Medaka as a research organism: past, present and future. Elsevier. Mechanisms of Development 121 (2004) 599-604.

15 ANNEX 1

Detailed study summaries for degradation, acute and chronic toxicity.

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Chemical Name: Dibenzoyl peroxide, benzoyl peroxide

EC Number: 202-327-6

CAS Number: 94-36-0

Index Number: 617-008-00-0

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Version number: 2 **Date: 23/11/2021**

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1 PHYSICAL HAZARDS

Not evaluated as part of this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated as part of this dossier.

3 HEALTH HAZARDS

Not evaluated as part of this dossier.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Study reference:

Anonymous (2015b), Biodegradability of Dibenzoyl peroxide (CAS No. 94-36-0) in the Closed Bottle Test (OECD 301D) (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type:

OECD Guideline Ready biodegradability Closed Bottle Test D (OECD 301 D). GLP compliant study. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Name:* Dibenzoyl peroxide. Identical to substance identified in CLH dossier.
- *Degree of purity:* 74.3%
- *Impurities*: Not reported.
- *Batch number:* Not reported.

Materials and methods:

- *Details on inoculum (nature and sampling site(s), concentration and any pre-conditioning treatment – any adaptation to be mentioned specifically)*: secondary activated sludge was obtained from the Nieuwgraaf wastewater treatment plant in Duiven (The Netherlands) which predominantly treats domestic wastewater. The activated sludge was preconditioned and 0.4 g dry weight (DW)/L of activated sludge was aerated for a period of one week.
- *Duration of test*: 28 days.
- *Details on test conditions (composition of medium, test temperature, pH, CEC (meq/100g), continuous darkness: yes/no, etc.)* Aerobic. Activated sludge, non-adapted. The nutrient medium of the study contained deionized water, monopotassium phosphate, dipotassium phosphate, disodium hydrogen

phosphate dihydrate, magnesium sulfate, calcium chloride, ferric chloride hexahydrate. Ammonium chloride was omitted from the medium to prevent nitrification. The pH of the media was reported to be 7.3 at the beginning of the test and 7.3 (controls) and 7.2 (test substance) at Day 28. The temperature ranged between 22 and 24°C. Bottles were closed and incubated for 28 days in darkness.

- *Details on test method:* Ten Biochemical Oxygen Demand (BOD) bottles of: inoculum only; dichloromethane treated inoculum (added and evaporated); test item (dibenzoyl peroxide) and inoculum; and six bottles of the reference substance (sodium acetate) were prepared and incubated for up to 28 days. The test was performed in 300 mL bottles with glass stoppers, completely filled and without air bubbles.
- *Identity of reference substance(s) used*: sodium acetate.
- *Test substance concentration, reference substance concentration:* The concentration of dibenzoyl peroxide and sodium acetate were 2.6 (2.0 active) and 6.7 mg/L, respectively.
- *Details on sampling (frequency, method and sterility)*: The oxygen content of the solutions was determined, in duplicate, at intervals of 0, 7, 14, 21 and 28 days using an oxygen electrode. The zero time bottles were immediately analysed for dissolved oxygen. The remaining bottles were closed and incubated for 28 days in the dark at constant temperature.
- *Details on analytical method to measure biodegradation:* The BOD of the reference and test substance solutions were calculated and compared to the calculated theoretical oxygen demand to determine the extent of degradation.
- *Parameter followed for degradation estimation*: oxygen consumption (% degradation).
- *Validity and deviations*: test validity fulfilled - endogenous respiration of 1.2 mg/L at Day 28; the differences of the replicate values at Day 28 were less than 20%; the percentage biodegradation of the reference compound, sodium acetate, was 91% at Day 14; and the oxygen concentrations remained >0.5 mg/L in all bottles over the test period. The robust study summary reports that there were no deviations from the OECD 301 guideline. However, the dossier submitter notes that secondary activated sludge was used as an inoculum instead of the recommended secondary effluent/surface water as per the OECD 301 guideline.

Results:

Under the conditions of the study, dibenzoyl peroxide reported a theoretical oxygen demand (ThOD) of 2.7 mg/L, corresponding to 71% degradation after 28 days. The study summary indicates that the 10-day window pass level was achieved with over 60% biodegradation reported in a period of approximately 10 days immediately following the attainment of 10% biodegradation. The reference substance, sodium acetate, was degraded by 91% after 14 days exceeding the pass-level threshold of ≥ 60% after 7 days for ready biodegradability, confirming the suitability of the inocula used. The rate of endogenous respiration reached 1.2 mg/L at Day 28 and the residual oxygen concentration remained above 0.5 mg/L (all test bottles) over the

duration of the test. Table 1 summarises the measured oxygen consumption (mg/L) and percentage of biodegradation (%) of dibenzoyl peroxide and the reference substance, sodium acetate.

Table 1 Oxygen consumption and percentage biodegradation of dibenzoyl peroxide and sodium acetate (reference substance) (Anonymous, 2015, ECHA dissemination site, 2021).

Time (days)	Oxygen consumption (mg/L)		Biodegradation (%)	
	Dibenzoyl peroxide	Sodium acetate	Dibenzoyl peroxide	Sodium acetate
0	0.0	0.0	0	0
7	2.2	4.3	58	80
14	2.4	4.9	63	91
21	2.7	-	71	-
28	2.7	-	71	-

The dossier submitter considers, under the conditions of this OECD 301 D Closed Bottle Test, dibenzoyl peroxide to be readily biodegradable (degradation 71% of the calculated biological oxygen demand after 28 days).

Study reference:

Anonymous (2009c): Dibenzoyl peroxide – Ready Biodegradability – Closed Bottle Test, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

OECD Guideline Ready Biodegradability Closed Bottle Test (OECD 301 D). GLP compliant study. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Name:* Dibenzoyl peroxide. Identical to substance identified in CLH dossier.
- *Degree of purity:* 74.6%
- *Impurities*: Not reported.
- *Batch number:* Not reported.

Materials and methods:

- *Details on inoculum (nature and sampling site(s), concentration and any pre-conditioning treatment – any adaptation to be mentioned specifically)*: Secondary activated sludge was obtained from a domestic wastewater treatment plant in the municipality Abidos, France. The test inoculum was prepared in the laboratory with activated sludge from the secondary effluent of the wastewater treatment plant. The robust study summary reports an inoculum bacteria concentration of between 10^7 and 10^8 cells per litre. The inoculum concentration in the test medium was equivalent to a maximum level of 30 mg/L (non-

adapted). The inoculum was pre-conditioned, by aerating the secondary effluent, without other treatment or addition, for 1 day at 20°C +/- 0.5°C.

- *Duration of test*: 28 days.
- *Details on test conditions and method:* Aerobic. Activated sludge, non-adapted. Details on the test method (e.g. number of samples, test apparatus, sampling frequency) are not reported in the study summary. The potential toxicity to the inoculum was investigated. An inhibition monitoring flask was prepared using equivalent quantities of test and reference substance, resulting in a theoretical oxygen mass of 1.33 mg per flask. The test solutions were inoculated with micro-organisms, and stored in closed, full bottles, away from light and at a constant temperature (20°C +/- 0.5°C) for 28 days. The pH was not reported in the robust study summary.
- *Identity of reference substance(s) used*: sodium benzoate.
- *Test substance concentration, reference substance concentration:* A 4 mg/L solution of dibenzoyl peroxide was prepared in dilution water and 150 mL used per flask (i.e. 0.6 mg), corresponding to a theoretical O_2 mass of 0.864 mg per flask (ThOD 1.44 mg O_2/mg). 108 mg of the reference substance, sodium benzoate, was dissolved in 100 mL of mineral medium. 1 mL aliquots of the solution was used per BOD bottle to give 1.08 mg per flask, corresponding to a theoretical O_2 mass of 1.804 mg per flask (ThOD 1.67 mg O_2/mg).
- *Method used to measure biodegradation:* The BOD of the reference and test substance solutions were calculated and compared to the calculated theoretical oxygen demand to determine the extent of degradation.
- *Parameter followed for degradation estimation*: oxygen consumption (% degradation)
- *Validity and deviations*: partially fulfilled – the study summary indicates that the oxygen depletion in the inoculum blank did not exceed 1.5 mg/L after 28 days and the residual oxygen concentrations remained > 0.5 mg/L in all bottles over the test period. However, the percentage of biodegradation reported for the reference compound, sodium benzoate, was 42% at Day 14 which is below the threshold of ≥ 60% after 7 days for ready biodegradability. The dossier submitter notes that secondary activated sludge was used as an inoculum instead of the recommended secondary effluent/surface water as per the OECD 301 guideline. The inoculum cell density (10^7 to 10^8 cells/litre) was higher than recommended by the OECD 301 guideline (10^4 to 10^6 cells/litre).

Results:

Under the conditions of the study, dibenzoyl peroxide degraded 68% by Day 28, exceeding the threshold for this test system. However, this level of biodegradation was not achieved within the required 10-day window. The reference substance, sodium benzoate, degraded by 42% after 14 days which is below the threshold of ≥ 60% after 7 days for ready biodegradability. The study authors attributed the reduced level of degradation to the activity of the inoculum which they considered not to be at its optimum level. The toxicity control,

containing both the reference substance and dibenzoyl peroxide, reported 42% biodegradation after 14 days. The OECD 301 D guideline indicates *" If in a toxicity test, containing both the test substance and a reference compound, less than 35% degradation (based on total DOC) or less than 25% (based on total ThOD or ThCO2) occurred within 14 days, the test substance can be assumed to be inhibitory (see Annex II for other toxicity tests)"*. As the reported degradation in the toxicity control is greater than 25% (ThOD) the test substance can be assumed to be non-inhibitory.

The dossier submitter considers that, under the conditions of the study, dibenzoyl peroxide demonstrated inherent biodegradability based on the observed biodegradation (68%) under the conditions of this Closed Bottle Ready Biodegradability test (degradation 68% of the calculated biological oxygen demand after 28 days).

Study reference:

Anonymous (1990): Biodegradability of Dibenzoyl peroxide, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

EEC/OECD Guideline Chapter 4 'Determination of Ready Biodegradability', comparable to the current OECD Guideline OECD 301 D Ready biodegradability (Closed Bottle Test). GLP compliant study. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Name:* Identical to the substance identified in the CLH dossier.
- *Degree of purity:* 74.4%
- *Impurities:* not specified.
- *Batch number:* not specified.

Materials and methods:

- *Details on inoculum (nature and sampling site(s), concentration and any pre-conditioning treatment – any adaptation to be mentioned specifically)*: Secondary activated sludge was obtained from the RZWI Nieuwgraaf, Duiven (The Netherlands) activated sludge plant which is reported to predominantly treat domestic wastewater. The test inoculum was prepared in the laboratory with activated sludge from the activated sludge plant. The activated sludge was preconditioned and 0.2g dry weight (DW)/L of activated sludge was aerated for a period of 6 days.
- *Duration of test*: 28 days (extended to 84 days).
- *Details on test conditions (composition of medium, test temperature, pH, CEC (meq/100g), continuous darkness: yes/no, etc.)* Aerobic. Secondary activated sludge. Ammonium chloride was omitted from the

nutrient medium to prevent nitrification. The pH of the medium was 7.4 at the end of the test period. Test temperature was not reported in the study summary.

- *Details on test method:* Stock solutions, 1000 mg/L, of sodium acetate (reference substance) and dibenzoyl peroxide (in dichloromethane) were prepared and added to 280 mL BOD bottles. Silica gel and dichloromethane were used to aid the application of the test substance into the BOD bottles. The study summary reports that silica gel control samples were incorporated into the study design to provide evidence that the presence of silica gel did not result in additional oxygen consumption. BOD bottles of the following were prepared and incubated for a period of 28 to 84 days:
  - mineral nutrient solution without test material and without inoculum (sample A);
  - mineral nutrient solution without test material but with inoculum (sample B);
  - mineral nutrient solution with test material (1.5 mg/L) on silica gel and inoculum (sample C);
  - mineral nutrient solution with sodium acetate (6.7 mg/L) and inoculum (sample D);
  - mineral nutrient solution without test material but with inoculum and silica gel (sample E); and
  - mineral nutrient solution without test material but with inoculum and evaporated silica gel (sample F).
- *Identity of reference substance(s) used*: sodium acetate.
- *Test substance concentration, reference substance concentration:* The concentration of dibenzoyl peroxide and sodium acetate in the BOD bottles were 1.5 and 6.7 mg/L, respectively.
- *Details on sampling (frequency, method and sterility)*: The electrode method was used to determine the dissolved oxygen concentration by using an oxygen electrode and metre. The study was prolonged to 84 days to measure the level of oxygen depletion of samples B, C and F using a funnel fitted in the BOD bottles. The oxygen electrode was subsequently placed in the BOD bottles to measure the oxygen concentration. The medium dissipated by the electrode was collected in the funnel. Following the withdrawal of the oxygen electrode, the collected medium was returned to the BOD bottle, the funnel was removed and the BOD bottle closed. The oxygen content of dibenzoyl peroxide was determined, using an oxygen electrode, at days 5, 15, 28, 42, 57 and 84. The oxygen content of sodium acetate was determined at days 5, 15 and 28. Duplicate measurements of all samples (A to F) were taken. The BOD of the reference and test substance solutions were calculated and compared to the calculated theoretical oxygen demand to determine the extent of degradation.
- *Measurement of biodegradation:* The BOD of the reference and test substance solutions were calculated and compared to the calculated theoretical oxygen demand to determine the extent of degradation.
- *Parameter followed for degradation estimation*: oxygen consumption (% degradation).
- *Validity and deviations*: validity criteria fulfilled - the rate of endogenous respiration reached 1.2 mg/L at Day 28 and the residual oxygen concentration remained above 0.5 mg/L (all test bottles) over the duration of the test; the percentage biodegradation of the reference compound, sodium acetate, was

94% at Day 14. The dossier submitter notes that although the validity criteria of the OECD 301 guidelines were fulfilled the following deviations from the test guideline were noted, which may affect the reliability of the study: Secondary activated sludge was used as the inoculum instead of the recommended secondary effluent (or surface water). Ammonium chloride was omitted from the medium to prevent nitrification. The testing intervals (5, 15 and 28 days) are different to those recommended in the current OECD 301 D guideline (7, 14, 21 and 28 days). The testing period was prolonged to 84 days. The OECD 301 D guideline recommends that for substances with a water solubility below 1 g/L, stock solutions are prepared in mineral medium or added directly to the mineral medium rather than in water/solvent as was performed in the study. A test concentration of 1.5 mg/L dibenzoyl peroxide was used in the study. In accordance with the general test conditions reported in Table 2 of the OECD 301 guideline, a test concentration of between 2-10 mg/L is recommended for the OECD 301 D test system. For insoluble substances, the OECD 301 D guideline recommends that bottles are periodically agitated during the incubation period to prevent falsely low degradation values. The robust study summary does not indicate that the bottles were agitated during the study and this may have had influenced the observed degradation rates. A number of the test conditions (e.g. the number of BOD bottles, test temperature, cell density and incubation conditions) were not reported.

Results:

Under the conditions of the study, dibenzoyl peroxide degraded 56% (ThOD) by Day 28, falling below the pass-level threshold of ≥ 60% ThOD for this test system (refer to Table 3 below). The reference substance, sodium benzoate, degraded by 81 and 88% after 5 and 15 days respectively. The rate of degradation was greater than the threshold for ready biodegradability (≥ 60% after 7 days).

Table 2 Mean measured oxygen concentration for all sample solutions (Anonymous, 1990, ECHA dissemination site, 2021).

Time (days)	Oxygen Concentration (mg/L)					
	A	B	C	D	E	F
0	9.1	9.1	9.1	9.1	9.1	9.1
5	8.8	8.6	8.9	4.4	8.7	8.4
15	8.7	8.4	8.2	3.8	8.0	8.1
28	8.6	8.2	7.0	3.3	8.0	8.0
42	-	8.1	6.1	-	-	7.6
57	-	7.9	5.6	-	-	7.2
84	-	8.2	5.6	-	-	7.1

Table 3 Oxygen consumption and percentage of biodegradation of the dibenzoyl peroxide and sodium acetate (reference substance) (Anonymous, 1990, ECHA dissemination site, 2021).

Time (days)	Oxygen consumption (mg/L)		Biodegradation (%)	
	Dibenzoyl peroxide	Sodium acetate	Dibenzoyl peroxide	Sodium acetate
5	1.1	4.2	39	81
15	1.5	4.6	52	88
28	1.6	4.9	56	94
42	1.5	-	52	-
57	1.6	-	56	-
84	1.5	-	52	-

Dibenzoyl peroxide was not considered readily biodegradable under the conditions of this Closed Bottle Ready Biodegradability test.

Study reference:

Anonymous, (1992): Biodegradation and Bioaccumulation Data of Existing Chemicals based on the CSCL. ECHA Dissemination site 2021.

Detailed study summary and results:

A non-GLP Ready Biodegradability Modified MITI Test (OECD 301 C). Not enough information reported in the study summary to evaluate the validity of the study.

Test substance:

- *Name*: Identical to the substance identified in the CLH dossier.
- *Degree of purity*: information not available.
- *Impurities*: Impurities do not affect the classification.
- *Batch number*: information not available.

Materials and methods:

The ready bioavailability of dibenzoyl peroxide was evaluated in a modified MITI test (OECD 301 C, 1992) for 21 days. Using an initial concentration of 100 mg/L dibenzoyl peroxide and 30 mg/L suspended solid (source unknown, non-adapted) a 300 mL test solution was prepared. An activity control (aniline in mineral medium at 100 mg/L with 30 mg/L activated sludge) and inoculum blank (mineral medium with 30 mg/L activated sludge) were also incorporated into the test design. Information on the test conditions and reference substance are not reported in the study summary.

Results:

Under the test conditions, dibenzoyl peroxide reportedly biodegraded by 83% (BOD), 88% (TOC), and 100% (HPLC analysis) after 21 days. The dossier submitter notes that the robust study summary concluded that dibenzoyl peroxide was readily biodegradable. However, there is no further information reported in the study summary on test design, conditions, results or validity criteria. The dossier submitter does not consider the study to be reliable.

4.1.2 BOD_5/COD

No data available.

4.1.3 Aquatic simulation tests

No data available.

4.1.4 Other degradability studies

Study reference:

Anonymous (2010d): Dibenzoyl peroxide Abiotic Degradation: Hydrolysis as a Function of pH (Preliminary Test), (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

Test performed in accordance with the OECD Guideline Hydrolysis as a Function of pH (OECD 111) and EU Method C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH. GLP compliant study. The OECD 111 Guideline indicates the preliminary study should be carried out for a period of 5 days. The robust study summary reports that this study was concluded after five hours due to the rapid rate of hydrolysis of dibenzoyl peroxide reported within this timeframe. Additional tier testing was not carried out as it was not considered warranted. This deviates from the OECD 111 guideline, which indicates that Tier 2 testing is to be undertaken for substances determined to be hydrolytically unstable in the preliminary study.

Test substance:

- *Name*: Identical to the substance identified in the CLH dossier.
- *Degree of purity*: 74.6%
- *Impurities*: not publicly available.
- *Batch number*: not publicly available.

Materials and methods:

The hydrolytic stability of non-radiolabelled dibenzoyl peroxide was studied in a preliminary OECD 111 Hydrolysis as a Function of pH study. Sterile aqueous buffer solutions at pH 4, 7 and 9 were treated with dibenzoyl peroxide and incubated, in the dark, at 50°C for 5 hours. Duplicate samples were analysed at 0, 2.4, and 5 hours (pH 4 and 7) and 0 and 0.5 hours (pH 9) by HPLC to determine the relative proportions of dibenzoyl peroxide and any degradation products. The pH of the solutions remained consistent throughout the study (refer to Table 4 below). The initial measured concentration of dibenzoyl peroxide was 0.15, 0.14, and 0.02 mg/L at pH 4, 7 and 9, respectively. Samples were analysed under modified HPLC conditions for benzoic acid, the expected main degradation product. The presence of benzoic acid was confirmed at each pH.

Table 4 Preliminary Hydrolysis Test - Measurements of pH (Anonymous 2010d, ECHA dissemination site)

Nominal	Initial pH	Final pH
4	4.2	4.2
7	7.1	7.1
9	9.1	9.0

Results:

Dibenzoyl peroxide was determined to be hydrolytically unstable at pH 4, 7 and 9 under the test conditions, with initial measured concentration of 0.15, 0.14, and 0.02 mg/L detected, respectively. The robust study summary reports that the preliminary study showed that greater than 50% hydrolysis had occurred after 2.4 hours at each pH (4, 7 and 9), equivalent to a half-life of less than 1 day under environmental conditions (25°C). The rate of hydrolysis increased with pH, to such an extent that only approximately 20% of the applied test substance was measureable at pH 9 on the initial sampling occasion. The principal hydrolysis product, benzoic acid, was detected in the sampled hydrolysed solutions at each of the tested pH. The dossier submitter notes that the available data for benzoic acid, as reported in the REACH registration dossier (ECHA dissemination site, 2021b), suggests that benzoic acid can be considered as readily biodegradable.

Dibenzoyl peroxide was determined to be hydrolytically unstable under acidic, neutral and basic conditions. The DT_{50} (25°C) is estimated to be < 1 day. The robust study summary indicates that no additional testing was performed.

Study reference:

Anonymous (2001c): The Test of Benzoyl peroxide Hydrolysis as a Function of pH. ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

A non-GLP test performed in accordance with the OECD Guideline Hydrolysis as a Function of pH (OECD 111). There is no further information reported in the robust study summary for the dossier submitter to verify the validity of the study or the observed results

Test substance:

- *Name*: Identical to the substance identified in the CLH dossier.
- *Degree of purity*: 70%
- *Batch number*: information not available.

Materials and methods:

The hydrolytic stability of non-radiolabelled dibenzoyl peroxide was studied in a preliminary OECD 111 Hydrolysis as a Function of pH study. Sterile aqueous buffer solutions of dibenzoyl peroxide (4 mg/L) at pH 4, 7 and 9 were prepared and incubated at 50°C for 5 days. To test for first-order behaviour, the test substance was analysed at pH 4, 7 and 9 and 25 °C by HPLC.

The dossier submitter notes that there is no further information on the study design, conditions and materials used reported in the study summary.

Results:

In the preliminary study, dibenzoyl peroxide reportedly degraded 93.5, 94.1 and 94.2% by Day 5 at pH 4, 7 and 9 and at 50 ºC, respectively. The half-life of dibenzoyl peroxide at pH 4 and 7 was determined to be 11.9 hours and 5.2 hours at 25°C, respectively. A half-life at pH 9 (and 25°C) could not be determined as dibenzoyl peroxide was not detected.

However, there is no further information reported in the robust study summary for the dossier submitter to verify the validity of the study or the observed results. The study is not considered reliable by the dossier submitter.

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

No data available.

4.2.2 Bioaccumulation test with other organisms

No data available.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

Study reference:

Anonymous (2010a): Dibenzoyl peroxide: Acute Toxicity to Fish, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

OECD Guideline 203 (Fish, Acute Toxicity Test), EU Method C.1 (Acute Toxicity for Fish), GLP compliant. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 74.6%
- *Impurities*: do not effect classification.
- *Physical state*: particulate powder.
- *Batch number*: not publicly available.

Materials and methods:

- *Test species and origin: Oncorhynchus mykiss* (Rainbow trout), obtained from a commercial fish farm in the UK.
- *Acclimation period:* 14 days.
- *Size and age of fish:* 6.14 cm (length), 3.04 g (mean wet weight); approximately 3 months.
- *Test conditions:* Semi-static; open glass aquaria containing 20 L of medium (18.5 cm liquid depth), aeration provided via narrow bore glass tubes; control: diluent water; hardness: 162-168 mg/L $CaCO_3$; dissolved oxygen: 65-103% (air saturation); pH: 7.97 – 8.43; temperature: 13.7 – 15.9°C; photoperiod: 16 hr light and 8 hr darkness. Fish were fed commercial fish food daily (1% of total wet-weight of fish in the holding tank). Fish were not fed for 20 hr before exposure, or during the 96 hr exposure period.
- *Test system:* semi-static, freshwater, daily renewal.
- *Tested doses*: nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg/L (geometric mean measured concentrations of 0.0081, 0.0097, 0.0224, 0.0316 and 0.0741 mg/L).

- *Sampling and sampling conditions:* 5 mL samples of media were taken from the control and test vessels at 0 and 48 hr (fresh media) and at 24 and 72 hr (expired media) for analysis. All samples were added to 0.2% acetic acid in acetonitrile (5 mL) in order to minimise further degradation of the parent material. On each occasion, one of the samples was analysed and the other was stored in a freezer in the event that further analysis was required.
- *Test duration:* 96 hr.
- *Test design:* 5 groups of 7 rainbow trout; 7 fish per vessel, 1.06 g bw/L initial static loading; test concentrations were measured throughout the test.
- *Observations:* Mortality and behavioural observations were made at 2 and 4 hr and every 24 hr following exposure.
- *Preliminary study:* Yes. Test concentrations of 1 and 10 mg/L. Results used to determine the conditions for the definitive study.
- *Validity criteria and deviations*: Validity criteria for the test guideline were met. No deviations reported.

Results:

The acute toxicity of dibenzoyl peroxide in rainbow trout was investigated under semi-static conditions for 96 hr. Hyperventilation was observed at the highest test concentration (10 mg/L nominal; 0.0741 mg/L mean measured) in 1 fish after 72 hr and 2 fish after 96 hr. No mortalities were observed. The dossier submitter notes that the actual (measured) exposure concentrations of dibenzoyl peroxide were significantly lower than the nominal concentrations used in the test, especially at the higher test concentrations. This, in part, may have been influenced by the low water solubility (0.35 mg/L), high absorptive potential (log Koc 3.8) and the unstable nature of dibenzoyl peroxide in water.

Under the conditions of the study, the 96 hr (semi-static) LC_{50} and NOEC of rainbow trout exposed to dibenzoyl peroxide were estimated to be 0.0602 mg/L and 0.0316 mg/L, respectively.

Study reference:

Anonymous (1989): Acute Toxicity of Dibenzoyl peroxide to fish, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test Type:

OECD Guideline 203 (Fish, Acute Toxicity Test), EU Method C.1 (Acute Toxicity for Fish), GLP compliant. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*:74.4%
- *Impurities*: do not effect classification.
- *Physical state*: white powder.
- *Batch number*: not publicly available.

Materials and methods:

- *Test species and origin: Poecilia reticulata* (Guppy), obtained from a local aquarium retailer.
- *Acclimation period:* 20 days.
- *Size and age of fish* Approximately 3 cm in length (outside the recommended length range of 1-2 cm. No rationale provided in the study summary for this deviation); life stage: not reported.
- *Test conditions:* Semi-static; covered glass aquaria; loading biomass: 0.6g biomass/L; control: deionised water (control I) and acetone (control II); solvent: acetone was used as an organic solvent to increase the solubility of the test substance; hardness: 13°dH; dissolved oxygen: 6.1-8.0 mg/L; pH: 8.2 (approx.); temperature: 24 – 24.5°C; salinity: no data; photoperiod:12 hr ambient light; food and feeding: not reported.
- *Test system:* semi-static, freshwater, renewed every 48 hr.
- *Tested doses*: nominal concentrations of 0.7, 1.3, 2.4 and 4.2 mg/L.
- *Sampling and sampling conditions:* Analytical monitoring of the test concentration was not performed. Measurements of the oxygen concentrations were conducted on days 2 and 4, while pH-measurements were conducted on days 0, 2 and 4.
- *Test duration:* 96 hr.
- *Test design:* 4 groups of 10 fish; 10 fish per vessel, 0.6 g biomass/L loading; test concentrations were not measured throughout the test.
- *Observations:* Mortality and behavioural observations were made at 24, 48, 72 and 96 hr.
- *Preliminary study:* Yes. The selected range of test concentrations was determined in a non-GLP preliminary range finding test with the following concentrations: 0.07 - 0.74 - 7.4 mg a.i./L.
- *Validity criteria and deviations*: Analytical monitoring of the test concentration was not performed. Considering this, the dossier submitter does not consider the validity criteria of the study to be fulfilled. In addition, the test organisms were obtained from a local aquarium retailer. This deviates from the recommendations of the test guideline. The length of the test organisms exceeded the recommendations of the test guideline. Based on the above deviations, the dossier submitter does not consider the study to be reliable.

Results:

The acute toxicity of dibenzoyl peroxide in guppy fish was investigated under aerated semi-static conditions for 96 hr. The numbers of surviving fish and the percentage mortality at the end of the test period are reported in Table 5.

Table 5 Number of surviving fish and percentage mortality following exposure to dibenzoyl peroxide (Anonymous 1989, ECHA dissemination site).

	No. Surviving Fish					Mortality at the end of test period (%)
Test Conc. (mg/L)	0 hr	24 hr	48 hr	72 hr	96 hr	
Control I	10	10	10	10	10	0
Control II	10	10	10	10	10	0
0.7	10	10	10	10	10	0
1.3	10	10	10	9	9	10
2.4	10	10	10	3	3	70
4.2	10	0*	-	-	-	100

* After 4 hours

In the highest treatment group, 4.2 mg/L (nominal), 100% mortality was observed after four hours. In addition, 10% (1/10) and 70% (7/10) mortality was reported in the 1.3 and 2.4 mg/L treatment groups after 48 hours, respectively.

Based on the conditions of the study, the LC_{50} and NOEC (96 hr) of guppy fish exposed to dibenzoyl peroxide, based on nominal concentrations, were estimated to be 2 mg/L (95% C.I. 1.7 and 2.4 mg/L) and 0.7 mg/L, respectively.

Study reference:

Anonymous (2002): The Acute Toxicity of Benzoyl peroxide to Fish. ECHA Dissemination site 2021.

Detailed study summary and results:

Test Type:

OECD Guideline 203 (Fish, Acute Toxicity Test), EU Method C.1 (Acute Toxicity for Fish), GLP compliant. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 97.3%
- *Impurities*: do not effect classification.
- *Batch number*: not reported.

Materials and methods:

- *Test species and origin: Oryzias latipes* (Japanese medaka), source: unknown.
- *Acclimation period:* 7 days.
- *Size and age of fish:* 3.5 cm ± 0.1 (outside the recommended length range of 1-2 cm. No rationale provided in the study summary for this deviation); 0.34 g (± 0.04 g); 9 months old.
- *Test conditions:* Continuous flow through; 8.7 L glass aquaria; control: diluent water (control I) and acetone (control II); hardness: 53.5 mg/L as $CaCO_3$ and alkalinity of 30.5 mg/L as $CaCO_3$; dissolved oxygen: 8.0-8.6 mg/L; pH: 7.27 – 7.55; temperature: 24.4-25°C; 16 hr light and 8 hr darkness (284-309 Lux light intensity). Fish were fed brine shrimp in the morning and Tetramin flake in the afternoon.
- *Test system:* continuous flow through, flow rate 167 mL/min, freshwater.
- *Tested doses*: nominal concentrations of 0.25, 0.5, 1.0, 2.0 and 4 mg/L (mean measured concentrations of 0.23, 0.47, 0.69, 1.54 and 2.17 mg/L).
- *Sampling and sampling conditions:* Analytical monitoring of the test concentration was performed at 0, 48 and 96 hr.
- *Test duration:* 96 hr.
- *Test design:* 5 groups of 10 Japanese medaka; 10 fish per vessel, test concentrations were measured throughout the test.
- *Observations:* Precipitation of the test substance was observed at the surface of the test medium at the 0.5 mg/L (nominal)/0.47 mg/L (mean measured) and 4 mg/L (nominal)/2.17 mg/L (mean measured) test concentrations. Mortality was recorded.
- *Preliminary study:* No.
- *Validity criteria and deviations*: Based on the information reported in the study summary, the dossier submitter considers that the validity criteria appear to be fulfilled. However, the source of the test species was not reported and the length of the test species deviated from the recommendations of the OECD 203 guideline (1 or 2 cm vs 3.5 cm). In accordance with the OECD 203 guideline, *'**test fish must be juveniles when used in this test (before reaching sexual maturity**). If fish of sizes other than those recommended are used, this should be reported together with developmental stage (juvenile, sub-*

adult, adult stage) and the rationale'. A rationale was not provided for using fish outside those recommended by the guideline. According to the literature, the body length of Japanese medaka at sexual maturity ranges from between 2.5 and 3 cm (Shiema et al., 2004). Based on the above deviations, the dossier submitter considers that the study is not reliable.

Results:

The acute toxicity of dibenzoyl peroxide in Japanese medaka fish was investigated under a continuous flow through test system for 96 hr. The mortality (number and percentage) observed from 24 to 96 hr are reported in Table 6.

Table 6 Number and percentage mortality following exposure of Japanese medaka fish to dibenzoyl peroxide (Anonymous, 2002, ECHA dissemination site).

Test Conc. (mg/L)		Mortality			
Nominal	**Mean Measured**	**24 hr No. (%)**	**48 hr No. (%)**	**72 hr No. (%)**	**96 hr No. (%)**
Control I	Control I	0 (0)	0 (0)	0 (0)	0 (0)
Control II	Control II	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0.23	0 (0)	0 (0)	0 (0)	0 (0)
0.5	0.47	2 (20)	6 (60)	10 (100)	10 (100)
1.0	0.69	4 (40)	10 (100)	10 (100)	10 (100)
2.0	1.54	10 (100)	10 (100)	10 (100)	10 (100)
4.0	2.17	10 (100)	10 (100)	10 (100)	10 (100)

The concentration of the test compound during the testing period exceeded 80 - 120% (testing guideline standard): 54.2 - 83.2 % of setting concentration at 0 hr, 101.3 - 105.3 % at 48 hr, and 101.3 % at 96 hr. No mortality was observed in the controls or the 0.23 mg/L (measured) test group at the end of the study period. 100% mortality was observed at 24 hr in the 1.54 and 2.17 (measured) mg/L test groups, at 48 hr for the 0.69 (measured) mg/L test group, and at 72 hr for the 0.47 (measured) mg/L test group.

Under the conditions of the study, the LC_{50} and NOEC (96 hr) of Japanese medaka exposed to dibenzoyl peroxide fish, based on mean measured concentrations, were estimated to be 0.24 mg/L (95% C.I. 0.20 and 0.27 mg/L) and 0.23 mg/L, respectively. Based on the deviations outlined above in the '*validity and deviations'* sub-section, the dossier submitter does not consider the study to be reliable.

Study reference:

Anonymous (1996): Establishment of Advanced Testing Methods for Hazardous Chemicals. ECHA Dissemination site 2021.

Detailed study summary and results:

Test Type:

OECD Guideline 203 (Fish, Acute Toxicity Test), GLP compliant.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: information not available.
- *Impurities*: do not effect classification.
- *Batch number*: information not available.

Materials and methods:

The acute toxicity of dibenzoyl peroxide in Japanese medaka (*Oryzias latipes*) fish was investigated in a non-GLP static test system, for 96 hr, in accordance with the OECD 203 Guideline Fish, Acute Toxicity Test. There is no further information reported in the study summary on the test species, test concentrations, conditions, and design.

Results:

The acute toxicity of dibenzoyl peroxide in Japanese medaka fish was investigated in a non-GLP static test system for 96 hr. The study summary reported an LC_{50} of 3.9 mg/L for Japanese medaka fish exposed to dibenzoyl peroxide for 96 hr. There is no information reported in the study summary on whether the test concentration was analytically measured. No further information is available on the study design, conditions, results and validity. The dossier submitter does not consider the study to be reliable.

4.3.2 Short-term toxicity to aquatic invertebrates

Study reference:

Anonymous (2010b): Dibenzoyl peroxide: Acute Toxicity to *Daphnia magna*, (Unpublished report). ECHA Dissemination site 2021. Refer to '*Materials and methods'* section for study deviations.

Detailed study summary and results:

Test Type:

OECD Guideline 202 (*Daphnia* sp. Acute Immobilisation Test), EU Method C.2 (Acute Toxicity for Daphnia), GLP compliant.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 74.6%
- *Impurities*: do not effect classification.

- *Batch number*: information not publicly available.

Materials and methods:

- *Test species and origin: Daphnia magna* (Planktonic crustacean); source: National Institute for Applied Chemical Research (IRCHA), France.
- *Species life stage:* < 24 hr old.
- *Test conditions:* hardness: 266 mg/L $CaCO_3$ (exceeded guideline recommendation); dissolved oxygen: 97 – 105 % (air saturation value); pH: 7.96 – 8.41; temperature: 19.4 – 21.0°C; photoperiod: 16 hr light and 8 hr darkness.
- *Test system:* static, freshwater
- *Test duration/total exposure duration:* 48 hr
- *Test substance preparation:* the test substance (20 mg) was dispersed in dilution medium (2 L) in a volumetric flask. The contents of the flask were stirred for approximately 16 hr (approximately 6 x the half-life), filtered through a pre-conditioned 0.2 µm nitrocellulose filter and then either used directly at the highest test concentration or diluted to provide the test media at the four lower concentrations.
- *Acclimation period:* -
- *Test design:* 20 neonate daphnids, 5 daphnids/replicate and 4 replicates per test concentration and control (diluent water), were exposed to dibenzoyl peroxide at nominal concentrations of 0.427, 0.939, 2.07, 4.55 and 10 mg/L (mean measured: <LOQ, < LOQ, 0.0416, 0.0765 and 0.157 mg/L); glass dishes, 20 mL medium per organism (biomass loading rate); test concentrations were measured at the beginning of the test. Test organisms were fed *Pseudokirchneriella subcapitata* during test (0.1 to 0.2 mg carbon per daphnid, per day, except during the initial 3 days when a slightly lower ration was given).
- *Sampling and sampling conditions:* analytical monitoring of the test substance, dibenzoyl peroxide and its main degradation product, benzoic acid was performed. 5 mL samples from each replicate flask for each group were taken at the beginning and end of the test. Samples were added to acetic acid (0.2 %) in acetonitrile, prior to quantitation, to minimise further degradation of the parent material. The concentration of dibenzoyl peroxide in the fresh samples was found to be between 0 – 2 % of the nominal concentrations in fresh samples and not detected in expired samples (48 hr). Benzoic acid concentrations were detected in the fresh samples, indicating that this was a degradation product of the test substance. Concentrations of benzoic acid in expired solutions were not detectable (< LOQ; 0.01 mg/L). Measured exposure concentrations of dibenzoyl peroxide were significantly lower than the nominal concentrations used in the test, especially at the higher test concentrations.
- *Effect parameters and observations:* Immobilisation
- *Preliminary study:* Yes. The selected range of test concentrations was determined in a preliminary range finding test with the following concentrations of dibenzoyl peroxide: 1, 10 and 100 mg/L. After

48 hr, immobilisation was observed at 10 and 100 mg/L while all daphnia in the 1.0 mg/L test were reportedly mobile.

- *Validity criteria and deviations*: Validity criteria for the test guideline were fulfilled. The dossier submitter notes that the hardness exceeded the upper limits recommended by the OECD 202 guideline. In addition, based on the information reported in the robust study summary, it is unclear if the test organisms were fed during the testing period. Feeding during the test period is not recommended in the OECD 202 guideline.

Results:

The acute toxicity of dibenzoyl peroxide to *Daphnia magna* was investigated under static conditions for 48 hours. The study summary reports, under the conditions of the study, an estimated 48 hr EC_{50} and NOEC of *Daphnia magna* exposed to dibenzoyl peroxide of 0.110 mg/L (95 % confidence limits of 0.0765 and 0.157 mg/L) and 0.0765 mg/L, respectively.

Study reference:

Anonymous (1999a): Effects of the Water Accommodated Fraction of Lucidol on the Growth of the Freshwater Green Alga *Pseudokirchneriella subcapitata*, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test Type:

Test was carried out in accordance with OECD (8.1) and EEC (8.2) guidelines for testing of chemicals and ECETOC, Monograph 26 (1996) (8.3), GLP compliant. Deviations reported below in the '*Validity criteria and deviations'* sub-section of the '*Materials and methods'* section.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 74.4%
- *Impurities*: do not effect classification.
- *Batch number*: information not publicly available.

Materials and methods:

- *Test species and origin: Daphnia magna* (Planktonic crustacean); source: from a continuous culture maintained at Akzo Nobel Chemicals, Arnhem, Dept. RGL.
- *Species life stage:* < 24 hr old.

- *Test conditions:* Hardness: 12 °dH; dissolved oxygen: 8.1 to 9.1 mg/L; pH: 7.7-8.0; temperature: 20.0 – 20.6°C; photoperiod: 16 hr light and 8 hr darkness (fluorescent tubes). Test medium was aerated before being used in the test. The air was water-saturated and purified by an active coal and cotton filter.
- *Test system:* static, WAF, freshwater.
- *Test duration/total exposure duration:* 48 hr.
- *Acclimation period:* -
- *Test design:* 20 neonate daphnids, 5 daphnids/replicate and 4 replicates per test concentration and control (deionised water), were exposed to WAF dilutions of dibenzoyl peroxide for a period of 48 hr. The WAF solutions were prepared from 2.0005g in 21 Dutch Standard Water (DSW) and filtered after 24 hr. This filtrate was subsequently used to prepare 1:2, 1:4, 1:8 and 1:16 WAF dilutions. An undiluted WAF sample and a negative control, containing only test medium, were also maintained under identical conditions but not exposed to the test item. Test chamber: glass dishes. Daphnids were not fed during the test period.
- *Sampling:* analytical monitoring measured by NPOC (non - purgeable organic carbon) at 0 hr and 48 hr.
- *Effect parameters and observations:* Immobility and sub-lethal effects recorded at 24 and 48 hr.
- *Preliminary study:* No.
- *Validity criteria and deviations*: Although the validity criteria of the study appear to be fulfilled, the dossier submitter does not consider the study to be reliable for the following reasons: the WAF method was used in the form of a series of dilutions. ECHA's *Information Requirements Chapter R.7b: Endpoint Specific Guidance (2017),* indicates that the WAF test method is generally used for substances that contain many constituents or for any substance with very low water solubility. It also indicates that all efforts should first be made to produce a reliable and stable test concentration, and only if this is not feasible, due to the properties of the substance or due to disproportionate efforts, can the WAF be considered as a last resort to generate exposure in a test. The guidance also indicates that the method used to prepare the WAF should be fully described in the test report, with evidence provided of attainment of equilibrium and its compositional stability over time if possible. It also stated that WAFs are to be prepared individually and not by serial dilution of a single WAF stock. The dossier submitter notes that the WAF dilutions were prepared from serial dilutions and monitoring was performed at the beginning and end of the test only. There is no further information reported in the study summary to indicate that the WAF method was the last resort. The reported effective test concentration was well above the water solubility of the substance. Finally, there is no further information reported in the robust study summary to determine if the test substance concentration was maintained throughout the study.

Results:

The acute toxicity of dibenzoyl peroxide to *Daphnia magna* was investigated via the WAF method under static conditions for 48 hr. The study summary reports that, under the conditions of the study, the estimated WAF EC_{50} and NOEC (48 hr) of dibenzoyl peroxide to *Daphnia magna* to be 2.91 mg/L (95% confidence limits of 2.71-3.11 mg/L) and 1.99 mg/L, respectively. Based on the deviations outlined above in the '*validity and deviations'* sub-section, the dossier submitter does not consider the study to be reliable.

Study reference:

Anonymous (2001a): The Acute Toxicity of Benzoyl peroxide to Aquatic Invertebrates (*Daphnia*). ECHA Dissemination site 2021. Refer to '*Materials and methods'* section for study deviations.

Detailed study summary and results:

Test Type:

OECD Guideline 202 (*Daphnia* sp. Acute Immobilisation Test), GLP compliant.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 79.4%
- *Impurities*: do not effect classification.
- *Batch number*: information not publicly available.

Materials and methods:

- *Test species and origin: Daphnia magna*; source: GSF Institute of Ecological Chemistry, Germany.
- *Species life stage:* 24 hr.
- *Test conditions:* Hardness: 226.5 mg/L ($CaCO_3$); Dissolved oxygen: 7.5-8.6 mg/L; pH: 8.0; Temperature: 21.0 – 21.1°C; photoperiod: photoperiod: 16 hr light and 8 hr dark (1427-1457 lux).
- *Test system:* static, freshwater.
- *Test duration/total exposure duration:* 48 hr
- *Acclimation period:* -
- *Test design:* 30 daphnids, 10 daphnids/replicate and 3 replicates per test concentration and control (dilution water), were exposed to dibenzoyl peroxide at nominal concentrations of 0.03, 0.06, 0.13, 0.25 and 0.5 mg/L for a period of 48 hr. A water control and solvent (acetone) control were also included and maintained under identical conditions but not exposed to the test item. Test chamber: 150 mL crystallizing dish. Information on food and feeding schedule were not reported. The concentration of the solvent control, acetone, exceeded 100 mg/L.
- *Sampling:* Analytical monitoring was performed by HPLC at 0, 1, 3 and 5 hr to identify the stability of dibenzoyl peroxide. The concentration of dibenzoyl peroxide was less than 80% after 1 hr. Due to rapid hydrolysis, nominal concentrations were used for calculating the EC_{50} value in the static system.

- *Effect parameters and observations:* Immobility and sub-lethal effects recorded at 24 and 48 hr.
- *Preliminary study:* No.
- *Validity criteria and deviations*: There is no further information reported in the study summary on the test design, conditions, results and validity. The dossier submitter does not consider the study to be reliable.

Results:

The acute toxicity of dibenzoyl peroxide to *Daphnia magna* was investigated under static conditions for 48 hr. The study summary stated that the response of the test organisms in the solvent control was normal. The study summary indicates that nominal concentrations were used to calculate the EC_{50} value in the static system due to rapid hydrolysis of the test substance. The observed immobilisation (number and percentage) of *Daphnia magna* following exposure to dibenzoyl peroxide at 24 and 48 hr are reported in Table 7.

Table 7 Cumulative Immobilisation (number and percentage) of *Daphnia magna* following exposure to dibenzoyl peroxide for 24 and 48 hr (Anonymous, 2001a, ECHA dissemination site, 2021).

Nominal Test Conc. (mg/L)	No. (%) immobilised organisms	
	24 hr	48 hr
Control	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)
0.03	0 (0)	0 (0)
0.06	0 (0)	3 (10)
0.13	30 (100)	30 (100)
0.25	30 (100)	30 (100)
0.50	30 (100)	30 (100)

As presented in Table 7, 100% immobilisation was observed in the 0.13, 0.25 and 0.50 mg/L test groups while 10 % immobilisation was observed in the 0.06 mg/L at 48 hours. The 48 hr EC_{50} of dibenzoyl peroxide to *Daphnia magna* was estimated to be 0.07 mg/L (nominal). Based on the considerations outlined above in the '*validity and deviations'* sub-section, the dossier submitter does not consider the study to be reliable.

4.3.3 Algal growth inhibition tests

Study reference:

Anonymous (2010c): Dibenzoyl peroxide Algal Growth Inhibition Assay, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

OECD Guideline 201 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test), EU Method C.3 (Algal Inhibition Test), GLP compliant.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 74.6%
- *Impurities*: do not effect classification.
- *Batch number*: information not publicly available.

Materials and methods:

- *Test species: Pseudokirchneriella subcapitata;* strain: CCAP 278/4; sourced from a culture collection of Algae and Protozoa (CCAP), SAMS Research Services Ltd., Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, Scotland.
- *Details on test organisms:* sterile algal nutrient medium was inoculated with cells aseptically removed from the slope culture. 100 mL of these primary liquid cultures were incubated for approximately 3 days in an orbital incubator under continuous illumination at nominal temperatures in the range 21 to 25°C. Subsequently, appropriate volumes of the primary cultures were aseptically transferred to fresh sterile algal nutrient medium to prepare secondary liquid cultures. These cultures were incubated for a further 3 days to provide an inoculum in the log phase of growth, characterised by a cell density of 0.847 x 10^6 cells/mL.
- *Test conditions:* test system: static, freshwater; test medium: standard algal nutrient medium; temperature: 21.9-22.8°C; pH: 7.45-7.65; hardness: not reported; dissolved oxygen: not reported; lighting: continuous illumination (4440 to 8880 lux).
- *Test duration/total exposure duration*: 72 hr.
- *Initial cell concentration*: 10000 cells/mL.
- *Control end cells density*: 261694 cells/mL (mean of 6), six replicates.
- *Test design:* test chamber*:* 250 mL conical flask, loosely stoppered with a foam bung covered with aluminium foil secured by autoclave tape. Flasks were sterilised before the start of the test. Test substance preparation: dibenzoyl peroxide (20 mg) was dispersed in dilution medium (2 L) in a volumetric flask. The contents of the flask were stirred for approximately 16 hours (approximately 6 x the half-life), filtered through a pre-conditioned 0.2 µm nitrocellulose filter and then either used directly at the highest test concentration or diluted to provide the test media at the four lower

concentrations. Test concentrations: nominal (as dibenzoyl peroxide) 0.427, 0.939, 2.07, 4.55 and 10 mg/L (0.034, 0.102, 0.166, 0.296, and 0.842 mg/L measured as benzoic acid), three replicates. Controls: the control cultures were prepared as per the test medium except that no test substance was added and a large volume (800 mL) of medium was made.

- *Sampling:* The test substance, dibenzoyl peroxide, and its main degradation product, benzoic acid, were measured via chemical analysis. At the start of the definitive test, two samples (5 mL) were taken from the freshly-prepared control and test media. After 72 hr, the contents of the replicate flasks for each group were pooled and further samples taken for analysis. Additional samples were also taken from a flask containing dibenzoyl peroxide at 0.427 and 10 mg/L but with no algal cells, in order to obtain information on the extent of adsorption/absorption of the test substance by the algal cells. All samples were added to 0.2 % acetic acid in acetonitrile (5 mL) in order to minimise further degradation of the parent material. On each occasion, one of the samples was analysed and the other was stored in a freezer in case further analysis was required.
- *Effect parameters:* determination of cell concentration via electronic particle count and size analyser.
- *Preliminary test:* Yes. A preliminary range finding study was performed with test concentrations of 1, 10, 100 mg/L (third test after deciding not to employ a solvent). The results used to determine the conditions for the definitive study. After 72 hr, algal growth was inhibited by 16 % at 1 mg/L, 97 % at 10 mg/L and 99 % at 100 mg/L.
- *Validity criteria and deviations:* Validity criteria considered fulfilled by the dossier submitter.

Results:

The potential growth inhibitory effects of dibenzoyl peroxide to freshwater algae (*Pseudokirchneriella subcapitata*) was investigated under static conditions for 72 hr. No microscopic abnormalities of the cells were detected. The levels of dibenzoyl peroxide in the fresh samples were found to be between 0 – 2 % of the nominal concentrations in fresh samples and not detected in expired samples (72 hr). Benzoic acid concentrations were detected in the fresh samples. Concentrations of benzoic acid in expired solutions were not detectable except in the two highest concentrations.

Under the conditions of the study the 72 hr E_bC_{50}, E_rC_{50}, E_yC_{50}, and NOEC (area under the growth curve, growth rate and yield) of algae exposed to dibenzoyl peroxide were 0.0422 mg/L, 0.0711 mg/L, 0.0724 mg/L and 0.02 mg/L respectively, based on initial measured concentrations in the fresh samples.

Study reference:

Anonymous (1999b): Acute Toxicity of Lucidol to *Daphnia magna*, (Unpublished report). ECHA Dissemination site 2021. Refer to '*Materials and methods'* section for study deviations.

Detailed study summary and results:

Test type:

The algal growth inhibition test was carried out in accordance with an OECD Guideline for testing of chemicals (9.1), which follows the EEC (9.2) and ISO Guidelines, (9.3) and with ECETOC Monograph 26 (1996) (9.4). Modifications and deviations: see '*Validity criteria'* below in section on '*Materials and methods*'. GLP compliant.

Test substance:

- *Substance*: Lucidol (Dibenzoyl peroxide).
- *Degree of purity*: information not available.
- *Impurities*: do not effect classification.
- *Batch number*: not reported.

Materials and methods:

- *Test species: Pseudokirchneriella subcapitata;* strain: CCAP 278/4; sourced from a culture collection of Algae and Protozoa, The Ferry House, Cumbria, Ambleside, United Kingdom (ISBN 1 871105056).
- *Details on test organism:* Cultures on sloped agar tubes were stored at 4°C until required. The initial stock culture was inoculated with *Pseudokirchneriella subcapitata* from a sloped agar tube and checked for purity by microscopic means. This algal stock culture (40 mL) of *Pseudokirchneriella subcapitata* was transferred regularly to fresh medium to act as inoculum for testing.
- *Test conditions:* static, freshwater. Test medium: mineral salts medium prepared from concentrated solutions of the mineral salts prepared in deionized water and stored at 4°C in the dark. A temperature-controlled illuminated orbital incubator was used as the culturing apparatus and the temperature maintained at 23°C ± 2°C. Continuous illumination was provided in the spectral range of 400 to 700 nm using 30 W fluorescent lamps (colour temperature of approximately 4000 K), at a distance of approximately 0.35 m from the algal cultures. pH: exact pH not reported. The robust study summary indicated the pH of all samples and controls were measured at the beginning and the end of the test and that the maximum variation in pH in the test media was 1.1 pH unit per test vessel; hardness: not reported; dissolved oxygen: not reported.
- *Sampling:* chemical analysis of the test concentration were performed using NPOC (non-purgeable organic carbon) analysis according to SOP K7 (9.12). The extinction in each Erlenmeyer was measured after 0, 24, 48 and 72 hr. The dossier submitter notes that NPOC analysis is not a specific method for the test compound and therefore the results can only be used as an indication of the concentration of the test material present. The calculation of the Lucidol concentrations were based on the ratio of the Lucidol molecular weight and carbon content (242,23/168) and the measured NPOC concentration at to corrected for the DSW control (4.66-2.08=2.56 mg/L), resulting in a concentration for the undiluted fraction of 3.73 mg/L Lucidol. From this value the dilutions were calculated.
- *Test duration/total exposure duration*: 72 hr.

- *Cell concentrations*: The robust study summary indicates that the cell density of the controls increased by at least a factor of 16 within 72 hr. No further information is included in the study summary for the dossier submitter to confirm this. The cell concentration was determined photometrically with a UVNIS Spectrophotometer. Measurements were carried out at 436 nm in a cuvette with a light path of 4 cm.
- *Initial cell concentration*: not reported.
- *Test design:* static; test system: 100 mL Erlenmeyer flasks containing 40 mL medium and closed with cotton wool stoppers. The test was carried out as a Water Accommodated Fraction (WAF). Test concentrations: a solution of the test substance of 9.6 mg in 1L algal medium was prepared in a stoppered flask. This solution was stirred for 24 hr and subsequently filtrated (Sartolab, 0.2 µm). The undiluted WAF preparation was diluted: 1:32 - 1:16 - 1:8 - 1:4 and 1:2. Six replicates.
- *Controls conditions*: the control cultures were prepared as per the test medium except that no test substance was added.
- *Effect parameters:* determination of cell concentration.
- *Preliminary test:* No.
- *Validity criteria and deviations*: based on the information reported in the study summary the dossier submitter does not consider it possible to determine if the validity criteria were fulfilled. In addition, the test concentrations were determined by NPOC analysis. The dossier submitter notes that NPOC analysis is not a specific method for the test compound and therefore the results can only be used as an indication of the concentration of the test material present. The $NaHCO_3$ concentration of the test medium was 150 mg/L instead of 50 mg/L, as recommended by the OECD/EEC Guidelines, in order to maintain a more constant pH during the test. Information on the exact pH is not reported in the study summary. The WAF method was used in the form of a series of dilutions. ECHA's *Information Requirements Chapter R.7b: Endpoint Specific Guidance (2017)*, indicates that the WAF test method is generally used for substances that contain many constituents or for any substance with very low water solubility. It also indicates that all efforts should first be made to produce a reliable and stable test concentration, and only if this is not feasible, due to the properties of the substance or due to disproportionate efforts, can the WAF be considered as a last resort to generate exposure in a test. The guidance also indicates that the method used to prepare the WAF should be fully described in the test report, with evidence provided of attainment of equilibrium and its compositional stability over time if possible. It also stated that WAFs are to be prepared individually and not by serial dilution of a single WAF stock. The dossier submitter notes that the WAF dilutions were prepared from serial dilutions and monitoring was performed at the beginning and end of the test only. There is no further information reported in the study summary to indicate that the WAF method was the last resort. Consequently, the dossier submitter does not consider the study to be reliable.

Results:

The concentrations used in the calculations are based on NPOC measurements determined at the beginning of the test after filtration. NPOC analysis is not a specific analysis for the test compound and therefore the results can only be used as an indication of the concentration as they were not actually measured. In addition, according to the robust study summary, this data should be considered with care based on the results of the NPOC concentration of the control at 0 and 48 hr.

Based on these indicative values the toxicity of these WAF's to exponentially growing *Pseudokirchneriella subcapitata* was determined over an exposure period of 72 hr. Based on the indicative concentrations of dibenzoyl peroxide for *Pseudokirchneriella subcapitata*, an E_bC_{50} and E_rC_{50} (0-72 hr) of 0.44 mg/L (0.31-0.62 mg/L 95% confidence limits) and 0.83 mg/L (0.59-1.13 mg/L 95% confidence limits) were determined, respectively. An indicative NOEC and LOEC of 0.12 mg/L and 0.23 mg/L were determined, respectively.

Study reference:

Anonymous (2001b): The Toxicity of Benzoyl peroxide to Aquatic plants (algae). ECHA Dissemination site 2021.

Detailed study summary and results:

Test type:

OECD Guideline 201 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test), EU Method C.3 (Algal Inhibition Test). GLP status: not reported. Refer to '*Materials and methods'* section for study deviations.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 79.4%
- *Impurities*: do not effect classification.
- *Batch number*: not publicly available.

Materials and methods:

- *Test species: Pseudokirchneriella subcapitata;* source: not reported.
- *Details on test organism:* information not available.
- *Test conditions:* static; test medium: details of the medium not reported; temperature 22-24°; pH: 7.45-7.78 (0 hr) and 7.45-8.01 (72 hr); hardness: 226.5 mg/L as CaCO3; alkalinity: 39.0 mg/L as CaCO3; dissolved oxygen: not reported; solvent: acetone.
- *Sampling:* HPLC monitoring was performed at 0 and 24 hr. The test substance was not detected after 24 hr.
- *Test duration/total exposure duration*: 72 hr.
- *Cell concentrations*: refer to Table 8 below.
- *Initial cell concentration*: information not reported.

- *Test design:* test concentrations: nominal concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/L dibenzoyl peroxide, diluted in acetone; replicates: not reported.
- *Controls and conditions*: a negative and solvent (acetone) control were used in the study. No further details are reported.
- *Effect parameters:* determination of cell concentration.
- *Preliminary test:* not reported
- *Validity criteria and deviations:* The dossier submitter does not consider it possible to determine if the validity criteria of the test were fulfilled based upon the level of information reported in the study summary. In addition, the concentration of dibenzoyl peroxide was not maintained over the course of the test period and was not detectable after 24 hr. The dossier submitter does not consider the study to be reliable.

Results:

The observed cell density and growth effects (growth rate, inhibition, and area under the curve) of *Pseudokirchneriella subcapitata* exposed to dibenzoyl peroxide for 72 hr are reported in Table 8.

Table 8 Cell density, growth (rate and inhibition) and Area under the Curve of *Pseudokirchneriella subcapitata* exposed to dibenzoyl peroxide for 72 hr (Anonymous, 2001, ECHA dissemination site, 2021).

Nominal Conc. (mg/L)	Cell Density (10^4 cells/mL)				Growth rate	% Growth rate	% Inhibition	Area under the Curve	
	0hr	24hr	48hr	72hr				% Relative Growth rate	% Relative Inhibition
Control	1.4	3.1	32	130	0.063	-	-	-	-
Solvent control	1.2	2.4	25	66	0.055	87.8	12.2	63.0	37.0
0.05	1.3	1.9	22	59	0.053	83.8	16.2	55.2	44.8
0.1	1.2	2.1	19	67	0.053	84.4	15.6	57.2	42.8
0.2	1.2	1.5	6.4	53	0.052	82.6	17.4	34.5	65.5
0.4	0.99	1.1	2.7	20	0.041	64.4	35.6	12.7	87.3
0.8	1.0	0.78	0.54	0.04	-0.013	0	0	0	100

Under the conditions of the study the 72 hr (nominal) E_bC_{50} and E_rC_{50} of dibenzoyl peroxide to algae, were estimated to be 0.07 mg/L and 0.44 mg/L, respectively.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

No data available.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No data available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No data available.

4.4.4 Chronic toxicity to aquatic invertebrates

Study reference:

Anonymous (2015a): Dibenzoyl peroxide (CAS No. 94-36-0): *Daphnia magna* Reproduction test, (Unpublished report). ECHA Dissemination site 2021.

Detailed study summary and results:

Test Type:

OECD Guideline 211 (*Daphnia magna* Reproduction Test). GLP compliant. No deviations reported.

Test substance:

- *Substance*: Dibenzoyl peroxide.
- *Degree of purity*: 74.2%
- *Impurities*: do not effect classification.
- *Batch number*: information not publicly available.

Materials and methods:

- *Test species and origin: Daphnia magna* (Planktonic crustacean);
- *Source:* the test was carried out using 1st instar *Daphnia magna* derived from in-house laboratory cultures. Adult daphnia were maintained in 150 mL glass beakers containing Elendt M7 medium in a temperature controlled room at approximately 20°C. The lighting cycle was controlled to give a 16 hr light and 8 hr darkness cycle with 20 minute dawn and dusk transition periods. Cultures were fed daily with a mixture of algal suspension (*Desmodesmus subspicatus*) and Tetramin® flake food suspension. Gravid adults were isolated the day before test initiation, such that the young daphnids produced overnight were less than 24 hr old. These young were removed from the cultures and used for testing.
- *Species life stage:* < 24 hr old.
- *Test conditions:* hardness: 216 to 282 mg/L $CaCO_3$ in the control and the highest surviving test group throughout the test; dissolved oxygen: 8.6 mg O_2/L; pH: reported as 'deviation < 1.1 in the control group'; temperature: 18-22°C; photoperiod: 16 hr light (513 to 607 lux) and 8 hr darkness with 20 minute dawn and dusk transition periods for 21 days. Each vessel was randomly assigned to a position in the laboratory. The robust study summary indicates that the dissolved oxygen concentrations, pH and temperature were recorded before and after each test media renewal, with the exception of Day 14 old media which were not recorded in error. Measurements were made on one replicate for each test

concentration. The temperature was also measured every hour in one replicate of the control. The water hardness of the control and the highest surviving test concentration in the fresh and old media was measured once per week.

- *Test system:* semi-static, freshwater.
- *Test duration/total exposure duration:* 21 days.
- *Test substance preparation:* Preliminary solubility work indicated that the test item was practically insoluble in water using traditional methods of preparation (e.g. ultrasonication and high shear mixing). A media preparation trial was conducted in order to determine the solubility of the test item under test conditions. A nominal amount of test item (20 mg) was dispersed in 2 litres of test water with the aid of sonication for 30 minutes. Undissolved test item was removed by filtration through a 0.2 µm Gelman Acrocap filter (first approximate 500 mL discarded in order to pre condition the filter) to give a 100 % v/v saturated solution. A series of dilutions were made from this saturated solution to give the required test concentrations of 3.2, 5.6, 10, 18 and 32 % v/v (nominal) saturated solution. The concentration and stability of the test item in the test preparations were verified by chemical analysis on days 0, 1, 6, 7, 13, 14, 20 and 21.
- *Test design: Daphnia magna* were exposed (10 replicates of a single daphnid per group) to solutions of the test item at nominal concentrations of 3.2, 5.6, 10, 18 and 32 % v/v saturated solution (corresponding to time-weighted mean measured test concentrations of 0.00062, 0.0011, 0.0016, 0.0028 and 0.0074 mg/L). A single daphnid was placed in 100 mL test preparation (Elendt M7 medium) in a 150 mL glass vessel covered with a plastic lid under for 21 days. The test solutions were renewed daily throughout the test. Feeding: each daphnid received approximately 5 to 10 µL of an algal suspension (*Desmodesmus subspicatus*) and approximately 10 to 30 µL of Tetramin® flake food suspension daily. Feeding was at a level of approximately 0.1 to 0.2 mg carbon/daphnid/day, dependent on the age and size, with the exception of Day 4 when the carbon/daphnid was only 0.075 mg in error. Equal amounts of food were given to each daphnid. The control group was maintained under identical conditions but not exposed to the test item.
- *Effect parameters and observations:* On a daily basis the numbers of live and dead of the "Parental" (P1) generation, the numbers of live and dead "Filial" (F1) daphnia and the number of discarded unhatched eggs were counted. The general condition and size of the parental daphnia was assessed and compared with the controls. The number of daphnia with eggs or young in the brood pouch was determined daily. An immobilization criterion for the young daphnids was considered to be inappropriate due to the large numbers of off-spring produced in the flasks. At the end of the test, the length of each surviving parent animal was determined.
- *Sampling and sampling conditions:* Analytical monitoring (HPLC/UV) was performed. Quantitative analysis was performed on water samples from the control and each surviving test group (replicates pooled). Samples of the fresh test preparations were taken on days 0, 6, 13 and 20 and of the expired

test preparations on days 1, 7, 14 and 21. All samples were analysed on the day of sampling with the exception of samples on days 6 and 13 which were stored frozen prior to analysis. Duplicate samples were taken and stored frozen for further analysis if necessary. The results were calculated based on the time-weighted mean measured test concentration only as a conservative analysis of the data. Where the determined concentrations were less than the LOQ, a value of half the LOQ was used.

- *Validity criteria and deviations:* Validity criteria for the OECD 211 guideline were fulfilled. Control mortality in the adult *Daphnia magna* was 0 %; the mean number of offspring produced per control adult was 116; and the coefficient of variation around the mean number of offspring produced per control adult was 13.5 %. The exact pH of the test substance groups was not reported in the robust study summary. It was stated that there was a 'deviation of < 1.1 of the pH of the control group'.

Results:

Analysis of the fresh test preparations on days 0, 6, 13 and 20 showed measured test concentrations to be less than the LOQ (determined to be 0.00028 mg/L to 0.0646 mg/L). A decline in measured test concentration of the aged test preparations on days 1, 7, 14 and 21 was observed to be less than the LOQ on all occasions with the exception of the 32 % v/v saturated solution on Day 1 where a measured concentration of 0.0013 mg/L was determined. It was therefore considered appropriate to calculate the results based on the time-weighted mean measured test concentration only in order to give a "worst case" analysis of the data. Where the determined concentrations were less than the LOQ a value of half the LOQ was used.

The effects of dibenzoyl peroxide on the reproductive output of *Daphnia magna* was investigated under semi-static conditions for 21 days. The percentage parental survival and total number of live young exposed to dibenzoyl peroxide for 21 days hours are reported in Table 9.

Table 9 Parental survival and total number of live young following exposure of *Daphnia magna* to dibenzoyl peroxide for 21 days (Anonymous, 2015a, ECHA dissemination site, 2021).

Nominal (%v/v saturated solution)	Mean Measured (TWA mg/L)	P1 Generation % Survival (mortality)	Total No. Live Young	Total No. Live Young ex. Replicates with Parental Accidental or Inadvertent Mortalities*	No. Live Young/Parent at start of test ex. Replicates with Parental Accidental or Inadvertent Mortalities*
Control	Control	100 (0)	1083	1046	116
3.2	0.00062	80 (20)	924	892	112
5.6	0.0011	89 (11)	1056	900	113
10	0.0016	90 (10)	862	862	96**

18	0.0028	50 (50)	593	593	59**
32	0.0074	50 (50)	363	363	36**

* Excluding Replicates with Parental Accidental and/or Inadvertent Mortalities

** Statistically significant difference (reduction) in the number of live offspring per adult compared to the control.

The robust study summary reports that the parent daphnia in Replicate 5 of the control and Replicate 8 of the 5.6 % v/v saturated solution test group died as a result of being damaged during the transfer to fresh media. These mortalities were considered to be accidental mortalities and were excluded from the statistical analyses and the results based on a reduced number of replicates. Information on the effects of the test item on the F1 generation is limited as the young are removed soon after liberation from the brood pouch. An assessment was performed at each media renewal: "filial" daphnids produced by all the test groups were in the same general condition as the young produced by the controls over the duration of the test with the exception of the young produced in the 18 and 32 % v/v saturated solution on Day 13 which were observed to be floating at the surface. Young were first produced in the control test group on Day 9 of the test. 21 unhatched eggs were observed in the 3.2 % v/v saturated solution on Day 12 and no dead young were observed in all control and treatment groups surviving to maturation.

Significant mortality (immobilization) occurred at test concentrations of 0.0028 and 0.0074 mg/L (mean measured) resulting in 50 % mortality in both test groups by Day 21 indicating a prolonged toxic effect following exposure of *Daphnia magna* to dibenzoyl peroxide. Lower levels of immobilisation (between 10 and 20%) were observed at the test concentrations of 0.00062, 0.0011 and 0.0016 mg/L. Throughout the test some of the parent daphnia in all test concentrations were observed as pale when compared to the control daphnia. The robust study summary reports that there were no statistically significant differences (P 0.05) between the control and each test group in terms of length of the daphnids after 21 days exposure to the test item. The results of the time to first brood, the time to production of first brood, and the average body lengths of the 1st generation surviving adults were not reported. After 21 days there were no statistically significant differences in the number of live offspring produced per adult between the control and the 0.00062 and 0.0011 mg/L test groups. The 0.0016, 0.0028 and 0.0074 mg/L test groups showed a statistically significant difference (reduction) in the number of live offspring per adult compared to the control after 21 days. A NOEC (reproduction) of 0.0011 mg/L (mean measured) was derived accordingly.

Under the conditions of the study, the EC_{10} (reproduction) of *Daphnia magna* exposed to dibenzoyl peroxide for 21 days was estimated to be 0.001 mg/L (95%C.I. 0.00010-0.0018) based on the time-weighted mean measured test concentrations. A NOEC (reproduction) of 0.0011 mg/L (TWA mean measured) was also

derived based on the statistically significant differences (reduction) in the number of live offspring per adult compared to the control after 21 days.

4.4.5 Chronic toxicity to algae or aquatic plants

See short-term toxicity, Section 4.3.3.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Not applicable.

5 REFERENCES

- ECHA (2021a): Dibenzoyl peroxide registration dossier (accessed August 2021) https://echa.europa.eu/registration-dossier/-/registered-dossier/15164
- Anonymous (1989): Acute Toxicity of Dibenzoyl peroxide to fish. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1999a): Acute Toxicity of Lucidol to Daphnia magna. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1990): Biodegradability of Dibenzoyl peroxide. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2015b): Biodegradability of Dibenzoyl peroxide (CAS No. 94-36-0) in the Closed Bottle Test (OECD 301D). (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1992): Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010d): Dibenzoyl peroxide Abiotic Degradation: Hydrolysis as a Function of pH (Preliminary Test) (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010b): Dibenzoyl peroxide: Acute Toxicity to *Daphnia magna*. (Unpublished report). ECHA Dissemination site 2021.

- Anonymous (2010a): Dibenzoyl peroxide: Acute Toxicity to Fish. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2010c): Dibenzoyl peroxide Algal Growth Inhibition Assay. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2015a): Dibenzoyl peroxide (CAS No. 94-36-0): *Daphnia magna* Reproduction test. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (2009c): Dibenzoyl peroxide – Ready Biodegradability – Closed Bottle Test. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1999b): Effects of the Water Accommodated Fraction of Lucidol on the Growth of the Freshwater Green Alga *Pseudokirchneriella subcapitata*. (Unpublished report). ECHA Dissemination site 2021.
- Anonymous (1996): Establishment of Advanced Testing Methods for Hazardous Chemicals. ECHA Dissemination site 2021.
- Anonymous (2001a): The Acute Toxicity of Benzoyl peroxide to Aquatic Invertebrates (*Daphnia*). ECHA Dissemination site 2021.
- Anonymous (2002): The Acute Toxicity of Benzoyl peroxide to Fish. ECHA Dissemination site 2021.
- Anonymous (2001c): The Test of Benzoyl peroxide Hydrolysis as a Function of pH. ECHA Dissemination site 2021.
- Anonymous (2001b): The Toxicity of Benzoyl peroxide to Aquatic plants (algae). ECHA Dissemination site 2021.
- Shima, A. and Mitani, H. (2004). Medaka as a research organism: past, present and future. Elsevier. Mechanisms of Development 121 (2004) 599-604.
- ECHA (2017): Guidance on the application of the CLP criteria.