

Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification
and labelling at EU level of

4,4'-isopropylidenediphenol; bisphenol A

EC Number: 201-245-8

CAS Number: 80-05-7

CLH-O-0000006910-75-01/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 public 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
8 October 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: 4,4'-isopropylidenediphenol; Bisphenol A

EC Number: 201-245-8
CAS Number: 80-05-7
Index Number: 604-030-00-0

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Version number: 2.0
Date: April 2019

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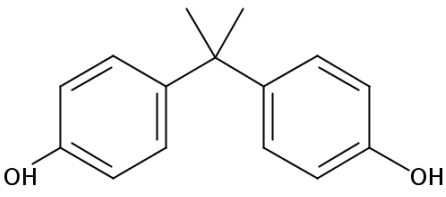
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1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4,4'-propane-2,2-diylidiphenol
Other names (usual name, trade name, abbreviation)	Bisphenol A
EC number (if available and appropriate)	201-245-8
EC name (if available and appropriate)	4,4'-Isopropylidenediphenol
CAS number (if available)	80-05-7
Molecular formula	C ₁₅ H ₁₆ O ₂
Structural formula	
SMILES notation (if available)	CC(C)(C1=CC=C(C=C1)O)C2=CC=C(C=C2)O
Molecular weight or molecular weight range	228.28 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	100 %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi- constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self- classification and labelling (CLP)
4,4'- <i>Isopropylidenediphenol</i> EC No: 201-245-8 CAS No: 80-05-7		see table 5	

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
-				

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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
-					

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	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	604-030-00-0	bisphenol A; 4,4'-isopropylidenediphenol	201-245-8	80-05-7	Repr. 1B STOT SE 3 Eye Dam. 1 Skin Sens. 1	H360F H335 H318 H317	GHS08 GHS05 GHS07 Dgr	H360F H335 H318 H317			
Dossier submitters proposal					Add Aquatic Acute 1 Aquatic Chronic 1	Add H400 H410	Add GHS09	Add H410		Add M (acute) =1 M (chronic) =10	
Resulting Annex VI entry if agreed by RAC and COM					Repr. 1B STOT SE 3 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360F H335 H318 H317 H400 H410	GHS08 GHS05 GHS07 GHS09 Dgr	H360F H335 H318 H317 H410		M =1 M =10	

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Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	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	Aquatic Acute 1 Aquatic Chronic 1	Yes
Hazardous to the ozone layer	not assessed in this dossier	No

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3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Bisphenol A has been classified under Dangerous Substance Directive (DSD, Directive 67/548/EEC) for environmental effects with R52 (30th ATP to DSD; Commission Directive 2008/58/EC). This classification was included in Annex VI Table 3.2 of CLP Regulation by 1st ATP (Commission Regulation (EC) No 790/2009). Nevertheless, no comparable criteria for R52 is available under CLP Regulation, therefore the classification was not included in Annex VI Table 3.1.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

Change in existing entry due to new data
Change in existing entry due to changes in the criteria
Differences in self-classification
Disagreement by DS with current self-classification

5 IDENTIFIED USES

Not relevant.

6 DATA SOURCES

Registration dossiers
Data from open literature
SVHC dossier (ECHA, 2017b)
Risk Assessment Report (European Commission, 2003; European Commission, 2010)

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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	Bisphenol A is a white solid at environmentally relevant temperatures	REACH lead registration dossier 2018	
Melting/freezing point	155 °C		
Boiling point	Boiling Point at 17 hPa: 250 - 252 °C (with potential decomposition), Boiling Point at 1013 hPa: 360 °C (with decomposition)		
Relative density	1.2 g/cm ³ at 25 °C		
Vapour pressure	4.12E-09 hPa at 25 °C		
Surface tension	Waiver		In accordance with column 2 of REACH Annex VII a study does not need to be conducted as, based on structure, surface activity is neither expected or predicted nor a desired property of the substance.
Water solubility	300 mg/L at 25 °C		
Partition coefficient n-octanol/water	3.4 at 21.5 °C and pH 6.4		
Granulometry	Waiver		In accordance with column 2 of REACH Annex VII, a study does not need to be conducted as the substance is marketed or used in a non granular form.
Stability in organic solvents and identity of relevant degradation products	Waiver		In accordance with column 1 of REACH Annex IX, a study does not need to be conducted as the stability of the substance is not considered to be critical.
Dissociation constant	11.3 at 20 °C		
Viscosity	Waiver		In accordance with section 1 of REACH Annex XI, a study does not need to be conducted as the substance is a solid at ambient temperatures.

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8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not assessed in this dossier.

10 EVALUATION OF HEALTH HAZARDS

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The reliability of all studies used was evaluated according to (Klimisch et al., 1997). The reliability of the studies not used in the Support Document for SVHC Identification of Bisphenol A (ECHA, 2017b) has been (re-)assessed by the dossier submitter as there is not reliability score mentioned in the Risk Assessment Report of Bisphenol A (European Commission, 2010).

11.1 Rapid degradability of organic substances

Table 8: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD 301 F	78.2 – 81.0 % O ₂ consumption after 28 days 76.3 – 81.2 % CO ₂ production after 28 days 10 day window fulfilled Reference substance: 98.3 % O ₂ consumption after 28 days 77.0 % CO ₂ production after 28 days (no data after 14 days mentioned)	Rel. 2	(West et al., 2001)
OECD 301 F	85 – 93 % O ₂ consumption after 28 days 10 day window fulfilled Reference substance: > 60 % biodegradation by day 14	Rel. 2	(Karagiri, 2004)
OECD 301 F	87.8 ± 6.9 % O ₂ consumption after 28 days 10 day window fulfilled Reference substance: > 60 % biodegradation by day 14 Toxicity control: > 25 % biodegradation by day 14	Rel. 2	(Stasinakis et al., 2008)
OECD 301 D	0 % O ₂ consumption after 28 days	Rel. 2	(European Commission, 2010) (Stone and Watkinson, 1983)
OECD 301 B	1-2 % CO ₂ evolution after 28 days	Rel. 2	(European Commission, 2010) (Stone and Watkinson, 1983)
OECD 301 C	0 % biodegradation after 14 days	Rel. 2	(MITI, 1992)
EPA OPPTS 835.3170 (shake flask die-away) Freshwater	20 °C ¹⁴ C-die away studies DT ₅₀ = 0.5-1.4 days 65-80 % ¹⁴ CO ₂ after 18 days	Rel. 1	(Klečka et al., 2001)

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Method	Results	Remarks	Reference
	Respirometer studies: DT ₅₀ = 0.5-2.6 days 59-96 % ThCO ₂ after 18 days		
River die-away test	30 °C DT ₅₀ = 2-3 days (primary degradation)	Rel. 2	(Kang and Kondo, 2002)
Shake flask die-away test Freshwater and seawater	Freshwater: 25 °C DT ₅₀ = 4 days (primary degradation) 35 °C DT ₅₀ = 3 days (primary degradation) Seawater: 25 °C and 35 °C: 80 % primary degradation after 60 days 4 °C: 30 % primary degradation after 60 days	Rel. 2	(Kang and Kondo, 2005)
Shake flask die-away test Freshwater	34 of 44 river water microcosms: 40-90 % removal after 14 days 6 of 44 river water microcosms: 100 % removal after 14 days 4 of 44 river water microcosms: 0 % removal after 14 days Metabolites: 2,3-bis(4-hydroxyphenyl)-1,2-propanediol and p-hydroxyphenacyl alcohol.	Rel. 2	(Ike et al., 2000)

11.1.1 Ready biodegradability

The ready biodegradability of Bisphenol A was evaluated in a manometric respirometry test (OECD 301F) at 22 °C (West et al., 2001). The initial concentrations of Bisphenol A used in this study were 7 and 25 mg/L (test material). The 7 mg/L concentration was used to evaluate biodegradation of Bisphenol A at the lowest concentration at which the respirometer could distinguish biodegradation of the test substance from background respirometer. As inoculum (30 mg/L suspended solid), activated sludge from a municipal wastewater treatment plant was used which was rated to be not adapted to the test substance. After a lag-phase of 4.7 days (7 mg/L Bisphenol A) and 5.2 days (25 mg/L Bisphenol A) the extent of biodegradation reached 78.2 to 81.0 % based on O₂ consumption and 76.3 to 81.2 % based on CO₂ production at day 28. 10 day window was met.

In a second OECD 301F test, Bisphenol A with an initial concentration of 100 mg/L and activated sludge (non-adapted, unknown concentration) from a municipal wastewater treatment plant was used (Karagiri, 2004). The lag phase ended in the time between day 7 and day 14. Bisphenol A was mineralised with 85-93 % (O₂ consumption) at day 28. 10 day window was met.

In a further OECD 301F test, Bisphenol A with an initial concentration of 35 mg/L (88.4 mg/L as ThOD) and 30 mg/L activated sludge from a municipal wastewater treatment plant was used (Stasinakis et al., 2008). In addition 10 mg/L allythiourea was added for preventing nitrification. After a lag phase of 4.3±0.3 days, Bisphenol A was mineralised with 87.8±6.9 % at day 28. Degradation reached 10 % by day 4.5 and exceeded 60 % by day 6.2.

The study of Stone and Watkinson (1983) was discussed in the Risk Assessment Report of Bisphenol A, which has been copied here in italic letters (European Commission, 2010):

Stone and Watkinson (1983) studied the biodegradation of Bisphenol-A in the OECD 301D Closed Bottle Test and the OECD 301B Modified Sturm Test. They also conducted an inhibition test on the growth of

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Pseudomonas fluorescens. The theoretical oxygen demand (ThOD) was calculated as 2.53 mg O₂/mg and the theoretical carbon dioxide evolution (ThCO₂) as 2.90 mg CO₂/mg.

In the Closed Bottle Test the initial test concentration used was 3 mg/L (test substance). The oxygen concentration in the bottles was measured at 5, 15, and 28 days. At the end of the test no degradation was observed. Inhibition of microbial activity was negligible under the test conditions.

In the Modified Sturm Test the initial concentration of bisphenol-A used was 20 mg/L (test substance). The test medium was dispensed into the Sturm vessels, inoculated and aerated with CO₂ free air. The extent of biodegradation was measured at 3, 7, 11, 18, 25, 27, and 28 days by titrating the total carbon dioxide released from the incubation. On day 27 the medium was acidified to release the total carbon dioxide by day 28. At the end of the test no degradation was observed.

In the microbial inhibition test the IC₅₀ for the inhibition of growth of *Pseudomonas fluorescens* by Bisphenol-A was 54.5 mg/L.

An OECD 301C test confirmed the results of Stone and Watkinson (1983). No biodegradation was observed after 14 days (MITI, 1992).

In the registration dossier further studies on ready biodegradability are available. Due to lack of information on experimental details these studies are not used for classification and labelling.

Conclusion on ready biodegradability:

If positive as well as negative results in ready biodegradability tests are available, then the data of the highest quality and the best documentation should be used for determining the ready biodegradability (ECHA, 2017a). Positive results could be considered valid (irrespective of negative results), when the scientific quality is good and the test conditions are well documented (e.g. guideline criteria fulfilled, non-adapted inoculum). Hence, based on above mentioned data Bisphenol A is readily biodegradable. Therefore, the substance is considered to be rapidly degradable for classification purposes.

11.1.2 BOD₅/COD

No data available.

11.1.3 Hydrolysis

The physical and chemical properties of Bisphenol A suggest that hydrolysis under environmental relevant conditions is negligible (European Commission, 2010).

11.1.4 Other convincing scientific evidence

No data available.

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 study with sufficient information on experimental design available.

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

The degradation of Bisphenol A was examined in surface water by Klečka et al. (Klečka et al., 2001). The water samples from seven different rivers across the United States and Europe were collected upstream and downstream from wastewater treatment plants known to treat wastewater containing Bisphenol A. Two different methods were conducted: River-die-away studies for ¹⁴C- Bisphenol A (initial Bisphenol A concentrations 50-5500 µg/L) and respirometry studies (initial Bisphenol A concentration 5000 µg/L). The

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test vessels were incubated at 20 ± 2 °C in the dark and were continuously stirred with 100 rpm. Bisphenol A was not detected in the river samples prior to the addition of the test compound. Negligible losses of Bisphenol A were observed in autoclaved controls, indicating no abiotic degradation. There was no significant difference between the tests conducted with different river waters or river waters upstream or downstream from wastewater treatment plants. The results indicated rapid biodegradation of Bisphenol A after an initial lag phase. In the ^{14}C river die-away studies lag periods of 2 - 8 days were observed and half-lives between 0.5 and 1.4 days were estimated. Degradation of ^{14}C - Bisphenol A resulted in mineralization with an average yield of 65-80 % $^{14}\text{CO}_2$ at the end of the test period (18 days). In the respirometry studies, after a lag period of 2.3-4.4 days, 59 - 96 % CO_2 was formed after 18 days. The estimated half-lives ranged from 0.5 to 2.6 days. In addition, the authors conducted studies with lower Bisphenol A concentrations (0.05 and 0.5 $\mu\text{g/L}$). Because of analytical limitations, only primary biodegradation was measured. After 28 days the Bisphenol A concentration was below 0.005 $\mu\text{g/L}$ and the estimated half-lives ranged from 3 to 6 days.

Kang and Kondo studied the primary degradation of Bisphenol A in river water (Kang and Kondo, 2002). Three river water samples were spiked with 1 mg/L Bisphenol A and incubated at 30 °C. In the river water samples no Bisphenol A was detected (LOD = 0.005 mg/L using HPLC analysis). Under aerobic conditions Bisphenol A was rapidly primarily degraded with half-lives of 2 - 3 days. After 10 days the concentration was below the LOD.

The same authors investigated primary degradation in seawater and in river water at different temperatures (25 °C, and 35 °C and additional 4 °C for seawater) (Kang and Kondo, 2005). The seawater samples were taken from five sites over one kilometer away from a junction of river and sea. The river water samples were collected from three rivers. All water samples were spiked with 1 mg/L Bisphenol A. In river water, half-lives were 4 and 3 days at 25 °C and 35 °C, respectively. In seawater lag periods of 30 days (25 °C and 35 °C) and 40 days (4 °C), respectively, were observed. At the end of the experiment (60 days) the initial concentration decreased to ~200 $\mu\text{g/L}$ at 25 °C/35 °C (80 % primary degradation) and ~700 $\mu\text{g/L}$ at 4 °C (30 % primary degradation). In autoclaved seawater, no degradation was observed over 60 days, indicating no abiotic removal process.

The biodegradation potential of Bisphenol A in 44 river water microcosms were investigated (Ike et al., 2000). The river water samples were collected from 15 sites of seven rivers with water quality ranging from clean to heavily polluted. A Bisphenol A solution was added to the systems to give a final TOC concentration of 20 mg/L. The systems were incubated at 28 °C with rotary shaking and dark conditions. 34 of the 44 river water systems showed TOC removal of 40 to 90 % after 14 days. Six microcosms could completely remove TOC and four microcosms showed no TOC removal within the test period. The removal of Bisphenol A in microcosms with unpolluted and less polluted river water is lower than in microcosms spiked with heavily polluted river water. Two metabolites were identified: 2,3-bis(4-hydroxyphenyl)1,2-propanediol and p-hydroxyphenacyl alcohol. These metabolites cannot be removed by Bisphenol A degrading bacteria.

Further studies on degradation of Bisphenol A in surface water are available in the registration dossier. All studies show rapid (primary) degradation of Bisphenol A in surface water.

Degradation data of Bisphenol A in water-sediment and soil do not need to be considered for the decision on rapid degradation, as Bisphenol A is demonstrated to be readily biodegradable based on available information from ready biodegradability tests ((ECHA, 2017a) Annex II.4 decision scheme).

Conclusion on surface water degradation data:

The available data demonstrate a rapid degradation of Bisphenol A in surface water once the system had become acclimated (lag-period).

11.1.4.4 Photochemical degradation

An atmospheric half-life of 0.2 days was calculated (AOPWIN) for the reaction of Bisphenol A with OH-radicals (European Commission, 2010).

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11.2 Environmental fate and other relevant information

The adsorption coefficients for environmental media were estimated using technical guidance document methods and a log Kow value of 3.40. The organic carbon-water partition coefficient (Koc) was estimated with a value of 715 L/kg. Several experimental studies observed a Koc value in the range of 251 to 1750 L/kg (outlier: 11,220 – 17,000). Bisphenol A is likely to be moderately adsorbed to solids (European Commission, 2010).

Volatilisation is not considered to be a significant removal mechanism for Bisphenol A from water systems (Henry's Law constant = $4.03 \cdot 10^{-6}$ Pa·m³·mol⁻¹) (European Commission, 2010).

11.3 Bioaccumulation

Table 9: Summary of relevant information on bioaccumulation

Method /Species	Results	Remarks	Reference
OECD 107	Log Kow 3.4	Rel. 2	(European Commission, 2010)
MITI guideline Carp (<i>Cyprinus carpio</i>)	BCF < 20 – 67.7 (15 µg/L) BCF = 5.1 – 13.3 (150 µg/L)	Rel. 1	(European Commission, 2003; European Commission, 2010)
Freshwater clams (<i>Pisidium amnicum</i>)	BCF = 144	Rel. 2	(European Commission, 2003; European Commission, 2010)
Killfish (<i>Oryzias latipes</i>)	BCF = 73.4	Rel. 2	(European Commission, 2010)

11.3.1 Estimated bioaccumulation

Not data available.

11.3.2 Measured partition coefficient and bioaccumulation test data

A log Kow of 3.4 was determined at 21.5 °C and pH 6.4 according to OECD Guideline 107.

Bioaccumulation was evaluated in the Risk Assessment Report of Bisphenol A, which has been copied here in italic letters (European Commission, 2010).

The available measured data suggested that bisphenol-A has a low potential for bioaccumulation in fish, in contrast to the moderate potential indicated by the log Kow value. A slightly higher potential was indicated by the measured bioconcentration in freshwater clams (up to 144). Measured data are preferred over calculated values when the studies are valid. A BCF of 67 for fish was therefore used in the published risk assessment, and the accumulation in clams was considered in the risk characterisation (EC, 2003).

The studies are specified in the Risk Assessment Report from 2003 and have been copied here in italic letters (European Commission, 2003).

*Bioconcentration factors for bisphenol-A have been measured by MITI (1977). Bioconcentration factors were determined for carp (*Cyprinus carpio*) exposed to bisphenol-A concentrations of 150 µg/L and 15 µg/L in a flow through system. The carp were exposed to bisphenol-A for six weeks. At the 150 µg/L exposure concentration bioconcentration factors of 5.1 to 13.3 were measured over the 6-week exposure period. At the 15 µg/L exposure concentration bioconcentration factors of <20 to 67.7 were measured over the 6-week exposure period. Bisphenol-A was judged to have a low bioaccumulation potential.*

*The accumulation of bisphenol-A in freshwater clams (*Pisidium amnicum*) has been studied at ecologically relevant low temperatures (Heinonen et al., 2002). Uptake and depuration rates were measured using ¹⁴C-labelled substance at temperatures between 2 and 12 °C. Both uptake and depuration rates increased with*

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temperature, although the uptake rate decreased slightly at the highest temperature. The bioconcentration factor was calculated from the concentration ratios at steady state and from the two rates. The maximum value was obtained at 8 °C by both methods, as 144 based on concentrations and 134 based on rates.

Information from 2003 to 2010 was also evaluated in (European Commission, 2010), but the new BCF values for fish are generally similar to that used in the published risk assessment and so no change is necessary. Two representative studies are described below.

Lindholm et al. (2003) studied the metabolism of bisphenol-A in zebrafish (Danio rerio) and rainbow trout (Oncorhynchus mykiss). Adult zebrafish were exposed to 100 µg/L bisphenol-A in a flow through system for 168 hours. Exposures took place in a 100 l aquarium, with a flow rate of 8 replacement volumes per day, and 150 fish. The bisphenol-A concentration was measured every two days; the actual concentration found was 97.5±5.2 µg/L. Fish were sampled to a system to which bisphenol-A was not added and kept for the same length of time, with sampling at the same intervals. Zebrafish tissue samples were analysed for Bisphenol A, Bisphenol A glucuronic acid (BPAGA) and Bisphenol A sulphate (BPAS).

Rainbow trout were exposed under similar conditions for eight days to 100 µg/L bisphenol-A (actual concentration from 2-day samples 107.3±6.3 µg/L). After eight days, gall bladder and blood samples were taken, and the bile fluid and blood plasma analysed for the same three substances (Bisphenol A, BPAGA and BPAS). Uptake and excretion rates for fish were calculated by fitting data to exponential uptake and decay models (much of the data for rainbow trout came from earlier publications). Uptake was fitted to a first order model, excretion to a first or second order model depending on the goodness of fit. Bisphenol A was detected in zebrafish after two hours' exposure, and steady state was reached by 24 hours. Steady state concentrations were 569 ng/g for Bisphenol A, 12.6 µg/g for BPAGA and 39.3 ng/g for BPAS. The whole body uptake rate for zebrafish was calculated as 0.23; tissue specific values from rainbow trout plasma, liver and muscle were 0.73, 0.11 and 0.16, so the rates were similar between the two species despite the different matrices. Elimination from zebrafish was fitted to a second order model; the first compartment had a half life of <1.1 hours, the second compartment half life was 139 hours. The three trout tissues had elimination half-lives of 3.7, 1.8 and 5.8 hours for plasma, liver and muscle respectively, as first order elimination. The authors suggest that in zebrafish Bisphenol A is rapidly removed from tissues, metabolised by the liver and excreted primarily as BPAGA into the gall bladder (compartment 2). Elimination from the tissues in zebrafish is much more rapid than from trout tissues. Zebrafish have a lower sensitivity to Bisphenol A than trout when considering vitellogenin synthesis. It is suggested that this may be due to the more rapid metabolism resulting in lower Bisphenol A concentrations and a reduced response. Data on specific tissue concentrations in the liver for Bisphenol A and metabolites was needed to confirm this.

Killifish (Oryzias latipes) were exposed to bisphenol-A at 17 µg/L in a flow-through system for six days (Takino et al, 1999). Fish were analysed at intervals, and the results at five and six days showed that steady state had been reached. The mean BCF from these two times was 73.4 l/kg.

Conclusion on bioaccumulation:

Bisphenol A has a low potential to bioconcentrate and is therefore not considered a bioaccumulative substance for classification purposes.

11.4 Acute aquatic hazard

There is much data available for short-term toxicity of BPA. Data was already evaluated in the Risk Assessment Report of Bisphenol A (European Commission, 2010) and briefly summarized in the Support Document for SVHC Identification of Bisphenol A (ECHA, 2017b). Further studies retrieved from literature dated beyond the last literature search for the Risk Assessment Report of Bisphenol A (European Commission, 2010) were also assessed. Most of them were evaluated and used in the Environmental Quality Standards (EQS) Dossier and also in the REACH registration dossiers.

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In the following table, the reliable studies relevant for classification purposes are listed with their respective observed effect concentrations (e.g. EC/LC₅₀). More detailed descriptions of the lowest relevant and reliable effect concentrations are provided in the following sections for fish, aquatic invertebrates as well as algae and aquatic plants.

Table 10: Summary of relevant information on acute aquatic toxicity*

Taxonomic group	Method	Species	Results [mg/L]	Remarks	Rel.	Reference
Fish	OECD 236	<i>Danio rerio</i>	96h-EC50= 5.25 96h-LC50= 8.04	Nominal	2	(Chan and Chan, 2012)
Fish	OECD 236	<i>Danio rerio</i>	72h-EC50= 13.8	Measured (analytical verification: HPLC)	2	(Duan et al., 2008)
Fish	No guideline	<i>Danio rerio</i>	120h-LC50= 5	Nominal (analytical verification: GC-MS); Embryos 3hpf; 25 replicates; experiment 3 times repeated	2	(McCormick et al., 2011)
Fish	OECD 203	<i>Oryzias latipes</i>	72h-LC50= 6.8 (adults)	Nominal (0.1 mL DMSO/L; no analytical verification); Flow-through test	2	(Kashiwada et al., 2002)
Fish	ASTM E-729-80	<i>Pimephales promelas</i>	96h-LC50= 4.6	Nominal (analytical verification: RPC)	1	(Alexander et al., 1988)
Fish	OECD 203/ GLP	<i>Cyprinodon variegatus</i>	96h-LC50= 11	.Measured	1	(Springborn Smithers Lab., 2009)
Fish	No guideline	<i>Cyprinodon variegatus</i>	96h-LC50= 7.5	Measured	2	(Dow Company, 1978) = (Emmitte 1978)
Fish	ASTM E729-80	<i>Menidia menidia</i>	96h-LC50= 9.4	Measured	1	(Alexander et al. 1988)
Crustacean	ASTM E729-80	<i>Daphnia magna</i>	48h-EC50= 10.2	Measured	1	(Alexander et al., 1988)
Crustacean	OECD 202	<i>Daphnia magna</i>	48h-EC50= 10.4	Nominal	2	(Jeong et al., 2013)
Crustacean	ISO 6341	<i>Daphnia magna</i>	24h-EC50= 13.8	Nominal, but measured	2	(Jemec et al., 2012)
Crustacean	OECD 202	<i>Daphnia magna</i>	48h-EC50= 9.9	Nominal (analytical verification: GC-MS)	1	(Mansilha et al., 2013)
Crustacean	No guideline	<i>Daphnia magna</i>	48h-EC50= 3.9	Nominal (no analytical verification); vehicle used	2	(Stephenson 1983)
Crustacean	OECD 202 (Daphtoxkit F magna)	<i>Daphnia magna</i>	48h-EC50= 10	Nominal	2	(Chen et al., 2002)
Crustacean	OECD 202 (Daphtoxkit F magna)	<i>Daphnia magna</i>	48h-EC50= 12.8	Nominal	2	(Hirano et al., 2004)
Crustacean	EPA-660/3-75-009	<i>Daphnia magna</i>	48h-EC50= 16	Nominal (no analytical verification); static	2	(Mu et al., 2005)
Crustacean	ISO 6341 15	<i>Daphnia magna</i>	48h-EC50= 7.75	Nominal (no analytical verification); static	2	(Brennan et al., 2006)

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Crustacean	No guideline	<i>Gammarus pulex</i>	120h-LC50= 1.5	Measured (GC-MS/MS); vehicle: > 0.5 % ethanol	2	(Watts et al., 2001)
Crustacean	ASTM E729-80	<i>Americamysis bahia</i>	96h-LC50= 1.1	Measured; Flow-through test	1	(Alexander et al., 1988)
Crustacean	EPA-600/4-90-027F	<i>Americamysis bahia</i>	96h-LC50= 1.03	n.a.	2	(Hirano et al., 2004)
Crustacean	Standard method developed at Artemia Reference Center (ARC-Test)	<i>Artemia franciscana</i>	48h-LC50= 34.7	Nominal	2	(Castritsi-Catharios et al., 2013)
Crustacean	No guideline.	<i>Tigriopus japonicus</i>	48h-LC50= 4.32	Nominal; vehicle used; semi-static	2	(Marcial et al., 2003)
Crustacean	ISO/DIS 14669	<i>Acartia tonsa</i>	72h-EC50= 0.96 (immobilization)	Nominal	2	(Andersen et al., 1999)
Crustacean	ISO 14669:1999	<i>Acartia clausi</i>	48h-LC50= 0.885	measured	1	(Tato et al., 2018)
Insect	EPA-540/9-85-005	<i>Chironomus tentans</i>	96h-LC50= 2.7	Measured	2	(Springborn Smithers Lab., 2005)
Mollusc	no guideline ("generally following OECD 203")	<i>Marisa cornuarietis</i>	96h-LC50= 2.24	Measured; semi-static; test at 25.3 to 25.5 °C	1	(Mihaich et al., 2009)
Echinoderm	No guideline	<i>Paracentrotus lividus</i>	72h-EC50= 0.71 (embryotoxicity)	Nominal; vehicle: DMSO; positive control: CdCl ₂	2	(Ozlem and Hatice, 2008)
Cnidaria	No guideline	<i>Hydra vulgaris</i>	96h-LC50 = 6.9	Measured; reference substance used	1	(Pascoe et al., 2002)
Platyhelminthes	ISO 6341	<i>Dugesia japonica</i>	48h-LC50= 8.3	Nominal; vehicle. 0.1 % DMSO	2	(Li, 2013)
Algae	EPA-600/9-78-010/ EPA-560/6-82-002	<i>Pseudokirchneriella subcapitata</i>	96h-EbC50= 2.73 96h-ErC50= 3.10	n.a.	2	(Alexander et al., 1988)
Algae	EPA-600/9-78-010/ EPA-560/6-82-002	<i>Skeletonema costatum</i>	96h-EbC50= 1.1	n.a.	2	(Alexander et al., 1988)
Algae	Similar to OECD 201	<i>Cyclotella caspia</i>	96h-ErC50= 7.96	Nominal (no analytical verification), vehicle: 0.50 % methanol	2	(Li et al., 2008)
Algae	Similar to OECD 201	<i>Navicula incerta</i>	96h-ErC50= 3.73	Measured (GC-MS)	2	(Liu et al., 2010)
Aquatic plants other than algae	OECD 221	<i>Lemna gibba</i>	7d-EC50= 20 mg/L (fond density)	Measured	1	(Putt 2003); (Mihaich et al., 2009)
Amphibian	No guideline	<i>Rhinella arenarum</i>	168h-LC50= 7.1	Nominal	2	(Hutler et al., 2014) (is the same as Wolkowicz et al., 2011)
Amphibian	No guideline	<i>Xenopus laevis</i>	72h-LC50= 4.8 (embryos)	Nominal	2	(Iwamuro et al., 2003)

* values in bold were also used for SSD

11.4.1 Acute (short-term) toxicity to fish

Short-term toxicity to fish was evaluated in the Risk Assessment Report of Bisphenol A (European Commission, 2010). There are additional studies available dating beyond the last literature search for the EU RAR. This data was evaluated for the CLH report. As these studies do not provide results lower than the one of 4.6 mg/L, they are listed in the table but not below in the text. The conclusions from the EU RAR have been copied here in italic letters (European Commission, 2010) as they are still relevant:

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For freshwater species the lowest acute toxicity value is a 96-hour LC₅₀ of 4.6 mg/L (nominal concentration) for the fathead minnow (*Pimephales promelas*). The test conditions and methods are fully described in the test report, and this test is considered valid.

For saltwater species the lowest acute toxicity value is a 96-hour LC₅₀ of 7.5 mg/L (measured concentration) for the sheepshead minnow (*Cyprinodon variegatus*). The test method used appears to be acceptable, although no information is given as to temperature, pH or dissolved oxygen during the test.

In summary, for fish the 96h-LC₅₀-value of 4.6 mg/L for *Pimephales promelas* is the lowest value for this trophic level.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Short-term toxicity to aquatic invertebrates was evaluated in the Risk Assessment Report of Bisphenol A (European Commission, 2010) for PNEC derivation. Some new data was added and is also listed in Table 10.

For crustaceans much data is available. According to the Guidance on the application of the CLP criteria (ECHA, 2017a) chapter 4.1.3.2.4.3 “where more than one acceptable test is available for the same taxonomic group, the most sensitive is generally used for classification. [...] When larger data sets (four or more values) are available for the same species, the geometric mean of toxicity values may be used as the representative toxicity value for that species. [...] This implies that for substances, where four or more ecotoxicity data on the same species and endpoint are available, the data should be grouped, and the geometric mean used as a representative toxicity value for that species.” The results for *Daphnia magna* (EC₅₀; 8 studies) range from 3.9 to 16 mg/L (geometric mean 9.47 mg/L calculated from 8 values). There is one study with *Artemia franciscana* and one with *Gammarus pulex* resulting in LC₅₀s of 34.7 and 1.5 mg/L, respectively. Two studies with *Americamysis bahia* provide similar results (LC₅₀s of 1.05 and 1.1 mg/L). One study with *Tigriopus japonicus* results in a LC₅₀ of 4.32 mg/L.

There are two studies with *Acartia* sp. with low effect concentrations. The one with *Acartia tonsa* results in a EC₅₀ of 0.96 mg/L. The other study was conducted with *Acartia clausii*, resulted in a LC₅₀ of 0.885 mg/L (measured concentrations) and is described in more detail below.

Tato et al. (2018) assessed the effects of the three phenolic compounds bisphenol A, triclosan and 4-nonylphenol to the marine organisms *Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Acartia clausi*. The experiments used 0.22µm-filtered sea water (34 ± 2 psu salinity, 8.2 ± 0.1 pH, 8.0 ± 0.1 mg/L dissolved oxygen). The acute lethal toxicity test with copepods followed standard methods (Water Quality. Determination of Acute Lethal Toxicity to Marine Copepods. ISO 14669:1999) using nauplius larvae. As reference substance for the biological quality of the stock 3,5-dichlorophenol was used. In total 40 vials per treatment were used. The vials were placed in an isothermal room at a temperature of 20 ± 1 °C and kept under an 18h light/6h dark photoperiod. The survival of the copepod was recorded after 48 hours of exposure. Stock solutions were prepared with dimethyl sulfoxide (DMSO) (0.01 %). The concentrations of Bisphenol A were analysed (DLLME-LC-MS/MS) with a limit of quantification (LOQ) of 20 ng/L. The measured concentrations ranged from 96.2 to 106.7 % (concentrations used: 150 – 300 – 600 – 1200 – 4800 µg/L). The exposure of *Acartia clausii* for 48h resulted in a NOEC of 300, a LOEC of 600 (44 % effect), an EC₁₀ of 186 and an EC₅₀ of 885 µg/L.

For the insect *Chironomus tentans* there is an LC₅₀ of 2.7 mg/L. For the mollusc *Marisa cornuarietis* the LC₅₀ is 2.24 mg/L. For echinoderms, the lowest acute toxicity value is an 96h-EC₅₀ of 0.227 mg/L for *Strongylocentrotus purpurus*. For cnidarians there is only one relevant study with *Hydra vulgaris* resulting in a LC₅₀ of 3.9 mg/L. And for the plathelminth *Dugesia japonica* a LC₅₀ of 8.3 mg/L is reported.

In summary, the 48h-LC₅₀-value of 0.885 mg/L for the crustacean *Acartia clausi* is considered valid and relevant for classification.

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

Short-term toxicity to algae and other aquatic plants was evaluated in the Risk Assessment Report of Bisphenol A (European Commission, 2010), which has been copied here in italic letters.

*Alexander et al. (1985b; 1988) report 96-hour EC₅₀ values, based upon cell count and total cell volume, of 2.73 mg/L and 3.10 mg/L for the green alga *Selenastrum capricornutum*, respectively. Both of the test results are based upon changes in biomass. The test report describes the test methods and test concentrations, and this test was considered valid for use in the PNEC derivation. In addition to the EC₅₀ values reported, the percentage inhibition of cell count and cell volume is reported for the concentrations tested. From these data it is possible to derive an EC₁₀ using probit analysis. The calculated 96 hour EC₁₀ values are 1.36 mg/L based upon cell count and 1.68 mg/L based upon cell volume.*

*Springborn Bionomics Inc. (1985c) (also published in Alexander et al. (1988)) report 96-hour EC₅₀ values, based upon cell count and chlorophyll content, of 1.0 mg/L and 1.8 mg/L, respectively for the marine alga *Skeletonema costatum*. The test report describes the test methods and test concentrations were measured. The method used to estimate the effect concentrations was non-linear interpolation. The percentage inhibition of cell count and chlorophyll content is reported for the concentrations tested. These original data have been analysed by the rapporteur [of the risk assessment report (European Commission, 2010)] using probit analysis in accordance with the OECD Guideline. The resulting EC₅₀ for cell count is 1.1 mg/L, and that for chlorophyll content is 1.4 mg/L. It is also possible to derive EC₁₀ values using the probit analysis. The calculated 96-hour EC₁₀ values are 0.69 mg/L based on chlorophyll content and 0.40 mg/L based upon cell count.*

*Stephenson (1983) reports a 96-hour EC₅₀ of 2.5 mg/L, based upon cell count, for the green alga *Selenastrum capricornutum*. The test report describes the test method used, however it does not give details of the test conditions. The test concentration is based upon nominal concentrations. This result should be used to support the data presented by Alexander et al. (1985).*

*Springborn Bionomics Inc. (1985c) (also published in Alexander et al. (1988)) report 96-hour EC₅₀ values, based upon cell count and chlorophyll content, of 1.0 mg/L and 1.8 mg/L, respectively for the marine alga *Skeletonema costatum*. The test report describes the test methods and test concentrations, and is considered valid for use in the PNEC derivation. However the method used to estimate the effect concentrations was non-linear interpolation. The percentage inhibition of cell count and chlorophyll content is reported for the concentrations tested. These original data have been analysed by (European Commission, 2010) using probit analysis in accordance with the OECD Guideline. The resulting EC₅₀ for cell count is 1.1 mg/L, and that for chlorophyll content is 1.4 mg/L. It is also possible to derive EC₁₀ values using the probit analysis. The calculated 96-hour EC₁₀ values are 0.69 mg/L based on chlorophyll content and 0.40 mg/L based upon cell count.*

Stephenson (1983) reports a 96-hour EC₅₀ of 2.5 mg/L (nominal), based upon cell count, for *P. subcapitata*. This result supports the data reported by Alexander et al. (1985b).

Liu et al. (2010) investigated the toxicity of BPA to the marine diatom *Navicula incerta* in a semi-static 96-h-test and estimated a EC₅₀ value for growth inhibition of 3.73 mg/L (analytically confirmed).

According to CLP guidance (ECHA, 2017a) the EC₅₀ value based on growth rate reduction is preferred. Therefore, in summary, the 96h-E_rC₅₀ of 3.73 mg/L for *Navicula incerta* is considered valid and relevant for classification.

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

There is an acute toxicity test for amphibians available, which was evaluated in the SVHC Support Document of Bisphenol A (ECHA, 2017b).

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The EC₅₀ for survival for *Rhinella arenarum* is 7.1 mg/L (Wolkowicz et al. 2014). This value is considered valid and relevant for classification.

Iwamuro et al. (2003) determined a 72h-LC₅₀ for survival for *Xenopus laevis* of 4.8 mg/L (nominal). About 60 to 100 embryos at stage 7 were exposed for 72 h to six different BPA concentrations, two E2 concentrations, or vehicle alone and then transferred to dechlorinated water. The number of surviving embryos was counted at 48, 96, and 120 h after the treatment.

11.5 Long-term aquatic hazard

There is much data available for the long-term toxicity of BPA. Data was already evaluated in the Risk Assessment Report of Bisphenol A (European Commission, 2010) and in the Support Document for SVHC Identification of Bisphenol A (ECHA, 2017b).

In the following table, the reliable studies relevant for classification purposes are listed with their respective observed effect concentrations (e.g. EC₁₀/NOEC/LOEC). More detailed descriptions of the lowest relevant and reliable effect concentrations are provided in the following sections for fish, aquatic invertebrates as well as algae and aquatic plants.

Table 11: Summary of relevant information on chronic aquatic toxicity*

Taxonomic group	Method	Species	Results ¹ [mg/L]	Endpoint	Reliability	Reference
Fish	Partial ELS test; No guideline; GLP	<i>Cyprinus carpio</i>	49d-NOEC= 0.1 (n)	Growth	2 (extended abstract, but already evaluated in EU RAR + considered suitable for PNEC derivation)	(Bowmer & Gimeno 2001)
Fish	FFLC EPA OPP 72-5 method developing study	<i>Danio rerio</i>	Full life-cycle 75dpf-NOEC= 0.75 (n)	Multiple end points growth, time to spawning, mating behaviour, eggs per female and fertilisation success	2	(Segner et al., 2003)
Fish	2-Generation-study; no guideline; conditions acc. to OECD 234	<i>Danio rerio</i>	300d-LOEC= 0.000372 (mean meas.) NOEC< 0.000372 (mean meas.)	Female biased sex ratio; malformation + mortality of larvae	2	(Chen et al., 2015)
Fish	No guideline; conditions similar OECD 234	<i>Danio rerio</i>	5 m-NOEC= 0.000174 (mean measured)	Reduced egg production	2	(Chen et al., 2017)
Fish	2-Gen.-study; no guideline; conditions acc. to OECD 234	<i>Danio rerio</i>	F2 180dpf NOEC= 0.2 (n) F0 90dpf-LOEC= 0.01 (= lowest conc. used)(n)	Growth	2	(Keiter et al., 2012)
Fish	No guideline	<i>Oryzias latipes</i>	14d-NOEC= 0.68	Reproduction	2	(Shioda and Wakabayashi, 2000)
Fish	OECD 210 (equivalent)	<i>Oryzias latipes</i>	60dph-NOEC= 0.355 (meas.)	Growth	2	(Yokota et al., 2000)

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Fish	Partial life-cycle test	<i>Oryzias latipes</i>	NOEC= 2.12 (mean meas.)	Growth	2	(Japanese Ministry of the Environment 2006)
Fish	Full life-cycle test	<i>Oryzias latipes</i>	NOEC= 0.247 (mean meas.)	F0 survival	2	(Japanese Ministry of the Environment 2006)
Fish	3-Gen.-study; No guideline	<i>Oryzias latipes</i>	Multi-generation LOEC= 0.084 (meas.)	Reduced embryo survival F3 generation	1	(Bhandari et al., 2015)
Fish	Similar to OECD 234	<i>Oryzias latipes</i>	44d- NOEC= 0.060 (n; analytically verified)	Reduced hatchability	2	(Sun et al., 2014)
Fish	Similar to OECD 230 (extended)	<i>Oryzias latipes</i>	21d-LOEC= 0.837 (meas.)	Gonadal histology	2	(Kang et al., 2002)
Fish	OECD 215 (proposal); GLP	<i>Oncorhynchus mykiss</i>	28d-NOEC= 3.64 (mean meas.)	Mortality	1	(Bayer AG 1999b)
Fish	FFLC EPA OPP 72-5 method developing study; GLP	<i>Pimephales promelas</i>	164d-NOEC= 0.16 (n, analytically verified)	Male survival	1	(ABC Laboratories, 2008); (Mihaich et al., 2012) = (Rhodes et al., 2007)
Fish	Similar to EPA OPP 72-5	<i>Pimephales promelas</i>	Full life-cycle 444d NOEC=0.016 (n, analytically verified)	Reduced egg hatchability F2 generation	2	(Brixham Environmental Labs, 2000a); (Brunel University, 2001); (Sohoni et al., 2001) = (Sumpter et al., 2001)
Fish	No guideline	<i>Salmo trutta</i>	5w-NOEC= 0.0024 (n)	Reproduction (no eggs)	2	(Lahnsteiner et al., 2005)
Fish	No guideline	<i>Salmo salar</i>	42d-NOEC= 0.1 (n)	Fry: lethargic, darker color, some with yolk sac oedemas	2	(Honkanen et al., 2004)
Fish	No guideline	<i>Poecilia reticulata</i>	30d-NOEC= 0.5 (n)	Survival	2	(Kinnberg and Toft, 2003)
Fish	OPPTS 850.1500	<i>Cyprinodon variegatus</i>	116d- NOEC= 0.066 (meas.)	F0 reproductive success	1	(Springborn Smithers, 2010)(Mihaich et al., 2018)
Crustacean	OECD 211	<i>Daphnia magna</i>	21d-NOEC \geq 3.146 (n.a.)	Reproduction	1	(Bayer AG, 1996); (Caspers, 1998)
Crustacean	ISO 10706	<i>Daphnia magna</i>	21 d NOEC 1.73 (n, analytically verified)	Reproduction	1	(Jemec et al., 2012)
Crustacean	OECD 211	<i>Daphnia magna</i>	21d NOEC 6.67 (n)	Reproduction	2	(Jeong et al., 2013)
Crustacean	OECD 211	<i>Daphnia magna</i>	21d NOEC 3 (n)	Reproduction	2	(Mansilha et al., 2013)
Crustacean	EPA-600/4-91/003	<i>Ceriodaphnia dubia</i>	7d-NOEC= 0.94 (n)	Reproduction	2	(Tatarazako et al., 2002)
Crustacean	ISO/DIS 14669	<i>Acartia tonsae</i>	5d-EC10=0.1 (n)	Development inhibition	2, No analytics	(Andersen et al., 2001)
Crustacean	No guideline	<i>Tigriopus japonicus</i>	21d-NOEC=0.001 (n)	Developmental delay (maturity) parental generation	2, no analytics	(Marcial et al., 2003)

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Crustacean	EPA 100.4	<i>Hyalella azteca</i>	42d-NOEC= 0.49	Reproduction	1	(Springborn Smithers 2006a); (Mihaich et al., 2009)
Crustacean		<i>Gammarus pulex</i>	14d-NOEC= 0.1 (n)	Survival (NOEC reproduction >1 mg/L)	2, conc. measured at start	(Johnson et al., 2005)
Crustacean	OPPTS draft 850.1350; GLP	<i>Americamysis bahia</i>	28d-NOEC= 0.17	Reproduction	1	(Lee 2010); (Mihaich et al., 2018)
Insect	No guideline	<i>Chironomus riparius</i>	Life-cycle NOEC= 0.1 (n, analytics: measured 83 % of nominal)	Time to larval moult + wet weight of 1 st instar larvae	2	(Watts et al., 2003)
Mollusc	No guideline; 25 °C	<i>Marisa cornuarietis</i>	181d/90d-NOEC= 0.0155 (meas., nom: 0.025 mg/L)	Adult fecundity/ Juvenile female growth,	1, GLP	(Forbes et al., 2007; Forbes et al., 2008); (Warbritton et al. 2007a+b)
Mollusc	No guideline; 20 °C	<i>Marisa cornuarietis</i>	150d-LOEC= 0.00025 (n), 0.000106 (med-meas., 0.000028 (twa) 150d-EC₁₀= 0.000038 (0.000053 twa, clutch)	Effects on egg / clutch production	2	Oehlmann et al. 2006, EC10 recalculation by (Ratte, 2015)
Mollusc	No guideline	<i>Potamopyrgus antipodarum</i>	90d-NOEC= 0.001 (n) 90d-NOEC= 0.025 (n)	Increased embryo prod. Decreased embryo p.	2, no analytics	(Jobling et al., 2004)
Mollusc	OECD detailed review paper on Molluscs life-cycle tox. testing	<i>Potamopyrgus antipodarum</i>	NOEC= 0.02 (n, 16 °C) 0.0194 meas NOEC=0.005 (n, 7 and 25 °C) 0.0046 meas)	Increased embryo prod.	2	(Sieratowicz et al., 2011)
Mollusc	No guideline	<i>Potamopyrgus antipodarum</i>	28d LOEC=0.0002 (n) 0.000168 (meas)	Increased embryo prod.	2, contaminate d controls	(Benstead, 2010)
Mollusc	No guideline	<i>Planorbis corneus</i>	56d-NOEC= 0.2 (n)	Reproduction	2	(Benstead, 2010)
Mollusc	Conditions acc. OECD 202	<i>Physa acuta</i>	21d-NOEC= 0.1	Reproduction	2	(Sanchez-Arguello et al., 2012)
Mollusc	No guideline	<i>Haliotis diversicolor</i>	8h NOEC=0.05 (n)	Embryo larval development	2	(Zhou et al., 2011)
Mollusc	No guideline	<i>Haliotis diversicolor</i>	EC10= 0.016 (n, but m)	Embryo development	2	(Liu et al., 2011)
Echinodermata	No guideline	<i>Paracentrotus lividus</i>	72h-NOEC= 0.0035 (n)	Larval malformations	2	(Özlem and Hatice 2008)
Echinodermata	No guideline	<i>Hemicentrotus pulcherrimus</i>	80d-LOEC=0.071 (n)	Suppressed juvenile growth	2	(Kiyomoto et al., 2005)
Porifera	No guideline	<i>Heteromyenia sp</i>	9d-NOEC= 1.6 (n, analytical verification of highest test conc.)	growth	2 used in RAR	(Hill et al., 2002)
Cnidaria	No guideline	<i>Hydra vulgaris</i>	6w-NOEC= 0.042 (n, analytically verified)	Growth, polyp structure	2 used in RAR	(Pascoe et al., 2002)
Cnidaria	No guideline	<i>Hydra oligactis</i>	50d-NOEC= 0.17 (n, analytically verified)	Reproduction	2 used in RAR	(Fukuhori et al., 2005)
Rotifer	No guideline	<i>Brachionus calyciflorus</i>	2d-NOEC= 1.8 (n.a.)	Rate of population increase	1	(Springborn Smithers 2006b); (Mihaich et al., 2009)

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Algae	EPA-560/6-82-002	<i>Pseudokirchneriella subcapitata</i>	4d-EC₁₀= 1.36 (n.a.)	Biomass	2	(Alexander et al. 1988)
Algae	EPA-560/6-82-002	<i>Skeletonema costatum</i>	4d-EC₁₀= 0.4	Biomass	2	(Alexander et al. 1988)
Macrophyte	OECD 221	<i>Lemna gibba</i>	7d-NOEC= 7.8	Growth	1	(Putt, 2003); (Mihaich et al., 2009)
Amphibian	No guideline	<i>Xenopus laevis</i>	120d-NOEC= 0.0073	Sex ratio (geomean of 1 st and 2 nd experiment)		(Levy, 2004; Pickford, 2010; Pickford et al., 2003)
Amphibian	No guideline	<i>Xenopus laevis</i>	90d-NOEC= 0.5 (n)	Mortality, growth, sexual differentiation	1	(Pickford, 2010; Pickford et al., 2003)
Amphibian	No guideline	<i>Xenopus laevis</i>	NOEC= 0.228 (n)	Abnormal development	2	(Baba et al., 2009)
Amphibian	No guideline	<i>Xenopus laevis</i>	21d-NOEC= 0.0228 (n)	T3-induced metamorphosis inhibition	2	(Heimeier et al., 2009)

¹ Results are based on the measured (no letter) concentration or nominal (n) or it is not applicable (n.a.); durations of the exposure phases are often given in the following way: days post-fertilisation (dpf); days post-hatch (dph); hours post-fertilisation (hpf); weeks (w); days (d)

* values in bold were also used for SSD (for *Daphnia*: geomean used = **3.23 mg/L**)

11.5.1 Chronic toxicity to fish

Chronic toxicity to fish was evaluated in the Risk Assessment Report (European Commission, 2010) and the SVHC Support Document of Bisphenol A (ECHA, 2017b). Citations from these reports are written in italic letters. Only the most sensitive reliable studies relevant for classification are described below in detail and are otherwise summarized for each species.

Carp (*Cyprinus carpio*)

For *Cyprinus carpio* Bowmer and Gimeno (2001) observed a 49d-NOEC for growth of 0.1 mg/L. Also effects on oviduct formation were observed in male carp in the same study (NOEC= 0.016 mg/L).

Zebrafish (*Danio rerio*)

Depending on the endpoint and study design the results (NOECs) for *Danio rerio* range from 0.000372 to 0.75 mg/L. The test design of these four studies differed, e.g. with test durations from 75 to 300 days. The lowest reliable effect concentration (LOEC) was observed by Chen et al. (2015) for the endpoints sex ratio, larval malformations and larval mortality and was already considered during SVHC identification with a female-biased sex ratio as clear endocrine mediated and adverse endpoint. This study is described in detail below (cited in italics from ECHA, 2017b):

Chen et al. (2015) investigated the effects of BPA exposure on Danio rerio (wild type AB strain) in a non-GLP two-generation study. It was a limit-test with a test concentration of 0.228 µg/L BPA (1 nM) (mean measured: 0.372 µg/L) and a solvent control (0.01 % DMSO) (mean measured BPA: 0.032 µg/L) (reliability 2). The exposure concentration was analytically confirmed by HPLC analysis in fresh but not in expired solutions (personal communication with the author Jiangfei Chen, November 2017: equal to (Chen et al., 2017)). The test was conducted semi-static at 28 °C (in the publication; personal communication of the registrant with the author: 22-25 °C) with 14 hours light per day as recommended in OECD Test guideline 234. The oxygen supply was obtained by using an air pump system. The precise oxygen saturation was not measured. Zebrafish embryos were obtained from spawning adults in tanks overnight with a sex ratio of 1:1. Embryos were collected within 0.5 h of spawning and rinsed in an embryo medium. They were used to start the first generation (F1) for the solvent control and BPA-exposure. Adult F1 fish (150 d) within the same treatment group were mated (4 females x 4 males/replicate; 3 replicates) to produce F2 embryos. Mating was conducted in clean water. The F2 embryos obtained from the F1 fish exposed to BPA were exposed at 8 hpf to BPA again (B2 – exposed for two generations), or not exposed (solvent only – exposed for one

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generation) (B1). There was also a group without exposure to BPA at all (B0). According to (Chen et al., 2015) the whole experiment was repeated three times each starting with a new batch of embryos (with a minimum of 90 embryos per replicate). The sex ratio was checked visually based on the morphological difference of the male and female zebrafish by an observer blind to the treatment. This was confirmed histologically on some fishes. Most were checked visually. There were no significant differences between the visually and histologically checked sex of fish (personal communication December 2017). The embryos were exposed from 8 hpf in a petri dish. After 8-72 hpf the water once was changed. 5 dpf the fish was moved to a 2 L tank. From 5 to 150 dpf the water was changed every 5 days. At day 21 post fertilisation the fish was moved to 9 L tanks. 90 eggs were used from each replicate to evaluate the embryo development. Chen et al. (2015) used 30 eggs per replicate. Four replicates were used for the first generation and three for the second generation. The sex ratio of control fishes was 57 % females (OECD 234: 30 to 70 % males or females). The statistical analysis was performed using an ANOVA followed by Tukey's multiple comparison test. For gene expression test, an unpaired t-test with 5 % FDR was performed. The control mortality was 2 to 16 %, the control fish body weight was 280 mg (mean) and the control length was 18 mm (mean). This is well in line with OECD 234 (<25 %). Chronic exposure to 1 nM BPA for only F1 generation or both F1 and F2 generation had no effect on adult fish survival nor were there any obvious malformations in parental F1 and F2 fish. The exposure resulted in a significantly altered sex ratio of the F1 and F2 population with more female in both F1 and F2 adults. BPA exposure also significantly reduced sperm counts and quality of F1 and F2 males (reduced sperm density, sperm motility, sperm ATP production, and significant increase in sperm lipid peroxidation). Despite the observation that BPA exposure reduced sperm density and quality, they found no evidence of histological lesions in testes of exposed fish. There were no significant differences in egg production and fertilisation of F1 and F2 females or adverse effects of embryo hatching or survival in offspring from F1 parents. The results fit well to the fact that sperm cells that successfully fertilized eggs are more likely to be normal in motility because only one sperm is required to fertilize one egg. Also with less motile sperm there is still enough to fertilise a typical spawn of eggs from the females. Paternal BPA exposure had a significant adverse effect on malformation (e.g., uninflated swim bladder, pericardial oedema and bent body) and mortality at 8 dpf. For example, % malformation in larvae derived from females paired with males from B0 was in a range of 7 ~ 17 %, which was increased to ~30 % in those paired with males from B1 and ~46 % in those paired with males from B2. Similarly, mortality increased from 2 % to 18 % in larvae derived from females paired with males from B0 to ~24 % and ~44 % in those paired with males from B1 and B2, respectively. It is possible that that this malformation and higher mortality results from an effect of BPA on sperm DNA. Chen et al. (2015) found reduced expression of *dnmt1*, *dnmt3*, *dnmt5* and *sp3* in 5 d old larvae which may contribute to the paternal-specific reproduction failure. This is similar to the result from a study with rats (Doshi et al., 2012). Chen et al. (2015) state that further studies are needed. In Chen et al. (2017) further effects of BPA exposure (nominal: 0.228, 2.28, and 22.8 µg/L) during different developmental stages (embryonic, larval, sexual mature) were examined. Exposure to 0.228 µg/L BPA during embryonic development increased malformations and mortality of offspring while egg production and fertilisation were reduced in higher concentrations (22.8 µg/L). Additionally, sperm quality (density, velocity, motility) and testis weight were decreased in F0 after embryonic exposure to 0.228 µg/L BPA.

Japanese medaka (*Oryzias latipes*)

For *Oryzias latipes* the No Observed (adverse) Effect Concentrations (NOECs) range from 0.060 to 2.12 mg/L, depending on the endpoint and test design.

Rainbow trout (*Oncorhynchus mykiss*)

For *Oncorhynchus mykiss* there is a 28-day juvenile growth test resulting in a NOEC for growth rate of 3.64 mg/L.

Fathead minnow (*Pimephales promelas*)

For *Pimephales promelas* there are studies with a range of NOECs from 0.016 to 0.16 mg/L, depending on the endpoint and test design.

Brown trout (*Salmo trutta*)

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For *Salmo trutta f. fario* Lahnsteiner et al. (2005) described a 103 d-study starting with male and female fish during late pre-spawning and spawning period. The approximately three years old wild caught brown trout were exposed via a flow-through test system to 1.75, 2.40 and 5.00 µg BPA/L (one tank per test concentration). DMSO was used with concentrations following OECD recommendations. There is no indication for a chemical analysis but due to the flow-through system and DMSO use, no loss of BPA is expected. Lahnsteiner et al. (2005) observed effects on egg production and semen fertility (LOEC= 5 µg/L; NOEC= 2.4 µg/L). At the highest exposure concentration (5.00 µg/L) no females gave eggs and the semen fertility was 28 %. They observed also a delay in the time point of ovulation (LOEC = 1.75 or 2.4 µg/L) but with only 6 fishes for this endpoint no significance can be proven and therefore is not used in the assessment (ECHA, 2017b).

Guppy (*Poecilia reticulata*)

For *Poecilia reticulata* there is a study observing effects of BPA after 30 days on survival above 0.5 mg/L (NOEC).

Sheepshead minnow (*Cyprinodon variegatus*)

There is 1.5-generation test available for *Cyprinodon variegatus* examining effects of 6 different BPA concentrations (control, 9.4, 19, 38, 75, 150, and 300 µg/L) in flow-through aquaria at 28 ± 1 °C. 50 embryos (< 30 hph) were placed into 28 incubation cups, with one cup in each of 4 replicate aquaria. On test day 4, 25 newly hatched fry in each incubation cup were placed in their respective growth chambers. At approximately 55 dph, spawning groups (2 males and 5 females) from each test aquarium were placed in one section of the corresponding spawning chamber in all test vessels, where they were held for 22 days. Exposure of F0 fish was terminated at 111 dph. Exposure of F1 fish was initiated by incubating groups of 50 embryos on the day they were spawned by placing them in the third section of the replicate tanks. Following hatching of the embryos, F1 exposure was continued by impartially placing a group of 25 newly hatched larvae (per replicate aquarium) into a corresponding larval growth chamber. The BPA concentrations were analytically confirmed (HPLC), resulting in measured concentrations of 76 to 88 % of the nominal ones (7.1, 17, 31, 66, 130, and 250 µg/L). Dissolved oxygen concentration and pH ranged from 5.1 to 8.3 mg/L (≥75 % saturation) and 6.7 to 8.2, respectively. Salinity ranged from 19 to 22‰, and exposure solution temperatures ranged from 26 to 29 °C. No effects were observed for larval survival or growth. The number of eggs per female per day was reduced, resulting in a NOEC of 0.066 mg/L.

Summary for fish

In summary, for classification the most sensitive fish species is *Danio rerio* with effects on sex ratio, larval malformations and larval mortality at 0.000372 mg/L (LOEC; measured concentration). The resulting NOEC is < 0.000372 mg/L (measured concentration). This test was assessed, discussed and taken into account for the Annex XV dossier for the identification of Bisphenol A as a substance of very high concern because of its endocrine disrupting properties (Article 57f) causing probable serious effects to the environment which give rise to an equivalent level of concern to those of CMR and PBT/vPvB properties, which was adopted on 14.12.2017 (ECHA, 2017b). The endpoint sex ratio is considered adverse. As this study used only one concentration (Limit-test), on which the described effects occurred, no definite NOEC could be derived. There are two other studies with very low effect concentrations conducted with *Dania rerio* (Chen et al., 2017; Keiter et al., 2012) – with a NOEC for egg production of 0.000174 mg/L and a NOEC for growth of < 0.01 mg/L, respectively – supporting this result.

11.5.2 Chronic toxicity to aquatic invertebrates

Chronic toxicity to aquatic invertebrates was already evaluated in the Risk Assessment Report of Bisphenol A (European Commission, 2010) as well as in the context of the identification of BPA as SVHC (ECHA 2017).

Crustacea

Daphnids were shown to be quite insensitive towards BPA with regard to chronic effect. This is shown in several studies with NOEC values between 1.7 and 6.6 mg/L for *Daphnia magna*. According to the Guidance on the application of the CLP criteria (ECHA, 2017a) chapter 4.1.3.2.4.3 “where more than one acceptable

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test is available for the same taxonomic group, the most sensitive is generally used for classification. [...] When larger data sets (four or more values) are available for the same species, the geometric mean of toxicity values may be used as the representative toxicity value for that species. [...] This implies that for substances, where four or more ecotoxicity data on the same species and endpoint are available, the data should be grouped, and the geometric mean used as a representative toxicity value for that species.” For *Daphnia magna* there are four toxicity values available and therefore the geometric mean was used, which is 3.23 mg/L. For *Ceriodaphnia dubia* there is a NOEC value of 0.94 mg/L available (see table). Copepods are more sensitive, but no studies with verified exposure concentrations are available although well-documented: an EC₁₀ of 100 µg/L for *Acartia tonsae* for larval developmental inhibition (Andersen et al. 2001) and 21d-NOECs of 0.1 (maturity parental) and **0.01 µg/L** (delay nauplii stage parental and maturity F1 generation). For the amphipod *Hyalella azteca* a NOEC for cumulative number of offspring per female was estimated as 490 µg/L by Mihaich et al. (2009) in a GLP-study using US EPA guidelines. For *Gammarus pulex* no effects on molting and reproduction could be discerned in concentrations up to 1000 µg/L (Johnson et al., 2005) without reporting results from analytics.

Insects

(Watts et al., 2003) observed a delay in time to the first molt and mean wet weights of first instar larvae at the highest test concentration (1000 µg/L). The actual concentration of the 1 mg/L test solution was measured to be 83 % of nominal concentrations and within 20 %. A NOEC of 0.1 mg/L was determined valid and was used in the RAR. Mouthpart deformities occurred at 0.01-100 µg/L (LOEC 0.01 µg/L), but the ecological significance is unclear.

Molluscs

Molluscs are a sensitive taxon with several valid results for the sexually reproducing *Marisa cornuarietis* and parthenogenetically reproducing *Potamopyrgus antipodarum*:

For *Marisa cornuarietis* (Oehlmann et al., 2006) reported results from two exposure series. While the first exposure series I (Oehlmann et al., 2000) and Schulte-Oehlmann et al. 2001) is not fully valid due to incomplete experimentation and only may be used as support, the second series II is valid. Both experiments used a strain originating from Aquazoo Düsseldorf with regular inbreeding and were conducted under a semi-static exposure regime, with medium renewal every day (every second day for weekends), a light:dark cycle of 12:12, acceptable water parameters and included a solvent control (12.5 µg/L Ethanol).

In exposure series II, 2 replicate groups of 30 sexually mature snails each were exposed to 0 - 0.25 - 0.5 - 1 and 5 µg/L BPA (additionally 5 µg/L bisphenol-A with either 3 µg/L of the anti-estrogen ICI 182 780 or 10 µg/L of the anti-estrogen tamoxifen) at 20 +/- 1 °C and 27 +/- 1 °C in parallel for 5 months (February-July). Samples for analytical determinations of exposure concentrations were taken over a 24-h period (before exchange of media until 24h later before next exchange of media) at month 1 for the second series (and month 1, 3 and 5 for the first series). Measured initial concentrations were very close to nominals, but concentrations declined between renewals. Results were therefore expressed in terms of median (not time-weighted) measured concentrations, which were between 39.0 % and 48.3 % of nominal levels. At 20 °C, BPA-exposed snails produced significantly more clutches and eggs compared to controls starting at the lowest test concentrations of 0.106 µg/L (measured, nominal: 0.25 µg/L). As a NOEC cannot be calculated, EC₁₀ values were estimated to be 0.0148 µg/L (95 % confidence interval 0.00607– 0.0362) and 0.018 µg/L (95 % confidence interval 0.062-0.525 µg/L) for egg and clutch production, respectively.

Similar effects were already observed in the first (incomplete) exposure series I at 22 °C starting from concentrations of 0.0079 µg/L (0.05 µg/L nominal) (calculated EC₁₀: 0.0139 respectively 0.0146 µg/L measured). Here, 210 mature snails were exposed in groups to 0 – 0.05 – 0.1 – 0.25 – 0.5 and 1 µg/L BPA and positive control 0.01 µg/L EE2, for 6 months (september- march) at 22 +/- 1 °C and samples of 30 snails collected every month for analysis.

At 27 °C in exposure series II, none of the treatment groups produced significantly more clutches, or eggs per female, than the control. A significant increase in egg production was only detected if measured in terms of cumulative egg number at 1 and 5 µg/ L (nominal) BPA. Here, the NOEC for egg production was 0.205 µg/L (measured, EC₁₀: 0.998 µg/L (95 % confidence interval 0.161-6.200 µg/L)) and the NOEC for clutch production was >1.990 µg/L (EC₁₀: 2.090 µg/L; 95 % confidence interval 0.796-5.460 µg/L).

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The temperature-related differences in NOECs are seen as a direct consequence of the lower egg production in controls observed at 20 °C (~500 eggs/female over the 5-month period). “Superfemales” with malformations of gonads (enlarged accessory glands, gross malformations and pallial oviduct) were already observed in the first exposure series at 22 °C at 1 µg/L (nominal). Also at 20 °C (series II), females with oviduct malformations were found with an incidence of 4.8 %, 8.0 %, 14.8 % and 11.5 % at 0.25, 0.5, 1, and 5 µg BPA/L (nominal), but not at 27 °C. Mortality was increased in groups experiencing oviduct malformation (around 10 deaths per treatment group at 20 °C, compared to 3 in the controls).

The study of (Oehlmann et al., 2006) is well-documented and reproduced at lower temperatures (20° and 22 °C). The effects on egg production were observed during a seasonal period of low spawning behaviour with a natural low egg production. The increased egg production is associated with malformations of gonads and increased mortality compared to controls at increasing test concentrations. Due to the statistical and analytical challenges, two subsequent attempts to recalculate effect concentrations have been made: (van der Hoeven, 2005) assumed a linear concentration response relationship and estimated a EC₁₀ of 2.1 µg/L (nominal), while Ratte (2009) estimated an **EC₁₀ of 0.038 µg/L (meas TWA)** for the parameter egg production based on measured concentrations. Definite effects with a **LOEC of 0.25 µg/L nominal (median measured 0.106 µg/L, TWA 0.028 µg/L)** for effects on egg and clutch production rate represent the “best case”, with effects potentially occurring even below and a NOEC certainly below.

Another study series with *Marisa cornuarietis* was conducted by Forbes et al. (2007a and b, Forbes et al. 2008) under a flow-through exposure regime and GLP standard for 181 days (6 months) using another strain originating from a tropical lake. The test method was previously established during a 12 month period without BPA exposure (see Auf der Heide et al. 2006 and Selck et al. 2006) and preliminary tests conducted at test concentrations of 0.1-1-16-160 and 640 µg/L at 25 °C for 3 months (see Warbritton et al. 2007a and Forbes et al. 2007a and b). Here no effects on egg hatchability, fecundity and growth rate could be discerned. In the definite test, nominal test concentrations of 0.1 – 1 – 25 and 640 µg BPA/L were applied using 3 replicates and tests were conducted at 25 °C. The evaluated endpoints comprised mortality, adult fecundity, hatchability and juvenile growth. The lowest NOEC was 25 µg/L nominal (measured 15.5 µg/L) both for juvenile growth (after 90 days) and for fecundity (after 181 days) at 25 °C. The results were confirmed in an additional experiment at 22 °C for one single test concentration (25 µg/L) after 84 days (see Forbes et al. 2008, Warbritton et al. 2007b). The study was conducted during seasonal conditions of high spawning behaviour with high/maximum egg production rates and may therefore have masked effects. It differs considerably with regard to test conditions from Oehlmann et al. 2006.

Sieratowitz et al. (2011) exposed *Potamopyrgus antipodarum* to 5 – 10 – 20 and 40 µg/L BPA for 28 days at different temperatures (7, 16, 25 °C). Chemical analyses were performed and experiments were conducted during pre-validation according to the test guideline proposal but examining temperature dependencies. At 16 °C a NOEC of 20 µg/L (19.4 meas), whereas at 7 and 25 °C **NOECs of 5 µg/L (meas: 4.6 µg/L)**, were estimated for the endpoint increased embryo production. The study is well-documented, valid and meets the quality criteria of the TG as conducted during pre-validation of TG 243.

Jobling et al. 2004 (corrected version) exposed *Potamopyrgus antipodarum* to 1 – 5 - 25 and 100 µg/L BPA nominal for 90 days in a semi-static system with 50 % of the dosed water being replaced every four days. Embryo production was significantly increased in comparison to controls after 63 days at 5 and 25 µg/L, while it decreased at 100 µg/L. A **NOEC of 1 µg/L (nominal)** was derived. As no analysis of the exposure solutions was performed, the study is not fully valid but supports a low NOEC for this species (as was also concluded by EU RAR).

Also Benstead et al. (2008), discussed in the transitional RAR of UK (ECHA 2009), report a significant increase in embryo numbers of *Potamopyrgus antipodarum* after 28d exposure at concentrations starting at the lowest test concentration of **0.2 µg/L (LOEC, nominal, measured: 0.168 µg/L)**. Test concentrations were 0.2 – 2 and 20 µg/L nominal. Hence, a NOEC could not be derived. Although the study had some drawbacks with respect to analytics (i.e. BPA was detected in the controls (5 times lower than test concentrations with effects), it supports stimulatory effects at low effect concentrations.

The observed effects on egg production in snails are supported by further studies with snails and are

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considered adverse. Also for the marine snail *Nucella lapillus* Oehlmann et al. (2000) observed an enhanced oocyte development and enlarged sex glands at low concentrations (NOEC < 1 µg/L nominal, no analytical confirmation). And for the marine sea snail *Haliotis diversicolor supertexta* Zhou et al. (2011) estimated a NOEC for embryo development of 0.05 mg/L. For the same species Liu et al. (2011) estimated an EC10 of 0.016 mg/L for embryo development and larval malformations and EC50s 0.18 and 1.02 µg/L for reaching trochophore stage and metamorphosis, nominal, but analytics confirmed -3+8 %). Benstead et al. (2010) reported an 56d NOEC of 0.2 mg/L for *Planeorbis corneus* on reproduction.

Further invertebrates (Echinodermata, Porifera, Cnidaria, Rotifera)

For echinoderms, an 72h-EC10 of 0.42 mg/L for *Paracentrotus lividus* (Özlem and Hatice 2008) and an 96h EC50 of 0.227 mg/L (Roepke et al. 2005) and a NOEC of 0.71 mg/L (Kiyomoto et al. 2006) for *Strongylocentrotus purpuratus* were reported.

Effects on the freshwater sponge (Porifera) *Heteromyenia* sp. were reported by Hill et al. (2002) and already considered in the EU RAR with a 9d NOEC of 1.6 mg/L (nominal, analytics for highest test conc.) with respect to growth.

For the cinidarian *Hydra vulgaris* a NOEC of 42 µg/L (nominal, analytics done) was estimated for the structure and physiology of polyps growth-related end point (Pascoe et al. 2002). For *Hydra oligactis*, a NOEC for suppression of testis formation of 500 µg/L is available (Fukuhori et al. 2005).

A 48-h NOEC of 1.8 mg/L (measured) is available for the rotifer *Brachionus calyciflorus* for the endpoint population increase (Mihaich et al., 2009)).

Summary for aquatic invertebrates

Snails are the most sensitive group, where valid results are available. The increase in egg production was observed in several experiments and at least two species. The LOEC of 0.25 µg/L nominal (median measured 0.106 µg/L, TWA 0.028 µg/L) for effects on egg and clutch production rate represents a reliable “best case”. Copepods are also sensitive, but no analytics were conducted.

11.5.3 Chronic toxicity to algae or other aquatic plants

Chronic toxicity to algae and other aquatic plants was evaluated in the Risk Assessment Report of Bisphenol A, which has been copied here in italic letters (European Commission, 2010).

Alexander et al. (1985b; 1988) [...] describes the test methods and test concentrations, and this test was considered valid for use in the PNEC derivation. In addition to the EC₅₀ values reported, the percentage inhibition of cell count and cell volume is reported for the concentrations tested. From these data it is possible to derive an EC₁₀ using probit analysis. The calculated 96 hour EC₁₀ values are 1.36 mg/L based upon cell count and 1.68 mg/L based upon cell volume.

*Springborn Bionomics Inc. (1985c) (also published in Alexander et al. (1988)) report 96-hour EC₅₀ values, based upon cell count and chlorophyll content, of 1.0 mg/L and 1.8 mg/L, respectively for the marine alga *Skeletonema costatum*. The test report describes the test methods and test concentrations were measured. The method used to estimate the effect concentrations was non-linear interpolation. The percentage inhibition of cell count and chlorophyll content is reported for the concentrations tested. These original data have been analysed by the rapporteur [of the risk assessment report (European Commission, 2010)] using probit analysis in accordance with the OECD Guideline. The resulting EC₅₀ for cell count is 1.1 mg/L, and that for chlorophyll content is 1.4 mg/L. It is also possible to derive EC₁₀ values using the probit analysis. The calculated 96-hour EC₁₀ values are 0.69 mg/L based on chlorophyll content and 0.40 mg/L based upon cell count.*

*Putt (2003) reports a 7-d frond density, biomass and growth rate NOEC of 7.8 mg/L for the duckweed *Lemna gibba*. The static-renewal study was performed to GLP according to OECD Guideline 221 and*

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analytical measurement of bisphenol-A showed that test concentrations remained between 79-100 % of nominal. The test report describes the test methods and test concentrations.

In summary, the 96h-EC₁₀ of 1.36 mg/L for *Ps.subcapitata* was considered valid in the risk assessment report and used in PNEC derivation for freshwater primary producers. For saltwater primary producers, the 96h-EC₁₀ of 0.40 mg/L for *S. costatum* was considered valid for the use in the derivation of a saltwater PNEC.

11.5.4 Chronic toxicity to other aquatic organisms

There are different chronic tests on toxicity to amphibians, which has been evaluated in the Risk Assessment Report and the SVHC Support Document of Bisphenol A (European Commission, 2010) (ECHA, 2017b).

The No observed effect concentrations (NOECs) range from 0.0073 mg/L to 0.5 mg/L for *Xenopus laevis* depending on test design and endpoint.

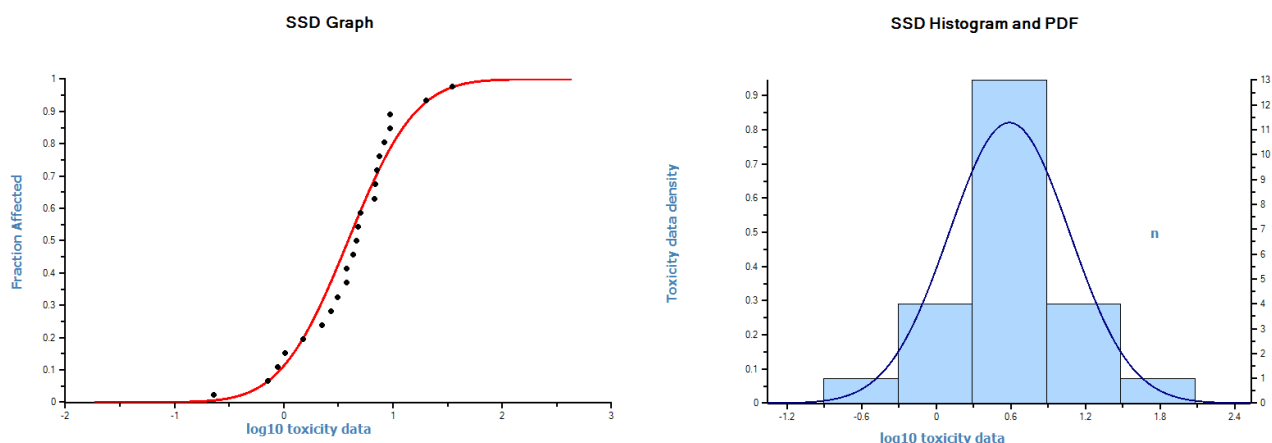
11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

The lowest valid EC₅₀ for classification is **0.885 mg/L** for the invertebrate *Acartia clausi* (Tato et al., 2018) (deterministic approach).

According to the CLP Guidance, in case of very large data sets according to ECHA Guidance R.10, statistical techniques (e.g. HC₅ derivation via a SSD) can be used to estimate the aquatic toxicity reference value for classification (equivalent to using the lowest EC₅₀), when the criteria for applying the Species Sensitivity Distribution (SSD) approach (probabilistic approach) are met. For BPA sufficient species (>10) and taxonomic groups (>8) are available to meet the criteria for applying the Species Sensitivity Distribution (SSD) approach. The acute freshwater and marine water ecotoxicity values displayed in bold in the table in section 12.4 above (table 10) were used for the SSD analysis. For *Daphnia magna* a geomean of 9.47 mg/L (nominal, 1 study measured) calculated from 8 studies was used for the 48h EC₅₀.

The SSD model assumes normal distribution of species sensitivities – for acute ecotoxicity this may be assumed as the endocrine mode of action of BPA rather exerts effects in long-term ecotoxicity studies. The data followed a normal distribution according to the three goodness-of-fit tests (Kolmogorov-Smirnov, Anderson-Darling and Cramer-von Mises).



“Goodness of fit” for the SSD acute EC₅₀ values for all taxonomic groups were calculated with the *E_TX* 2.1 software (Van Vlaardingen, 2014).

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Anderson-Darling test for normality			
Sign. level	Critical	Normal?	
0.1	0.631	Accepted	
0.05	0.752	Accepted	AD Statistic: 0.46223129
0.025	0.873	Accepted	n: 23
0.01	1.035	Accepted	

Kolmogorov-Smirnov test for normality			
Sign. level	Critical	Normal?	
0.1	0.819	Accepted	
0.05	0.895	Accepted	KS Statistic: 0.68502791
0.025	0.995	Accepted	n: 23
0.01	1.035	Accepted	

Cramer von Mises test for normality			
Sign. level	Critical	Normal?	
0.1	0.104	Accepted	
0.05	0.126	Accepted	CM Statistic: 0.07925451
0.025	0.148	Accepted	n: 23
0.01	0.179	Accepted	

A HC₅ of **0.60 mg/L** was obtained with lower and upper limits of 0.29 and 1.01 mg/L, respectively.

This value is very similar to the value derived using the deterministic approach based on the lowest effect concentration, i.e. the most sensitive species EC₅₀ of **0.885 mg/L**. Both values are below 1 mg/L and lead to a classification as Aquatic Acute 1 with an M-factor of 1.

Table 12: Comparison with criteria for acute aquatic hazards

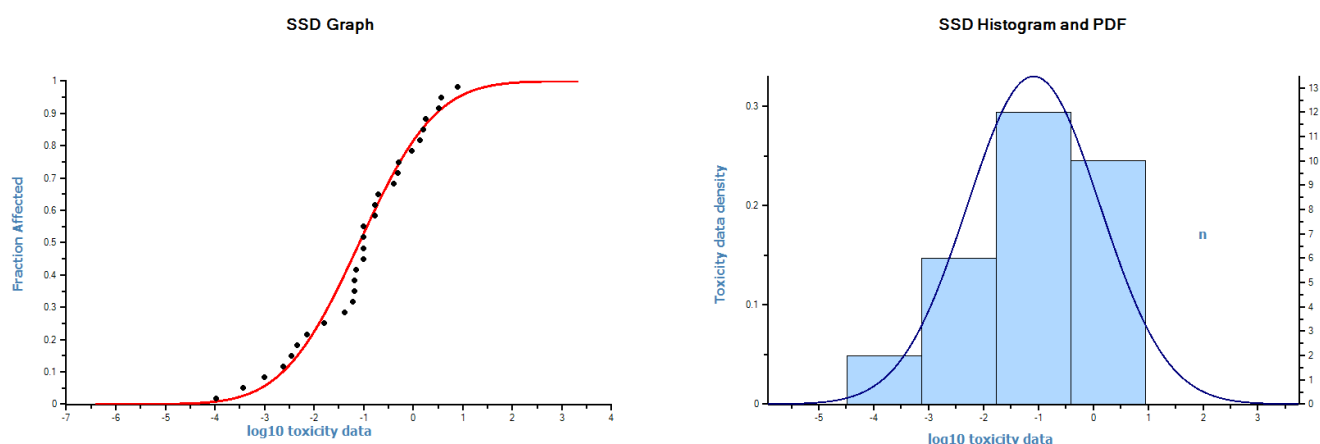
	Criteria for acute environmental hazards	Bisphenol A	Conclusion
Acute Aquatic Toxicity	Cat. 1: LC ₅₀ /EC ₅₀ /ErC ₅₀ ≤ 1 mg/L	Fish: 96h-LC ₅₀ = 4.6 mg/L (n) (<i>Pimephales promelas</i>) Invertebrates: 48h-LC ₅₀ = 0.885 mg/L (m) (<i>Acartia clausi</i>) Algae: 96h-ErC ₅₀ = 3.73 mg/L (m) (<i>Navicula incerta</i>) SSD: HC ₅ = 0.60 mg/L	Aquatic Acute 1, M= 1

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

For the most sensitive species in some studies effects occurred even at the lowest test concentrations. Therefore, not for all studies definite NOECs were determinable. It would not be appropriate to take only the studies into account, in which these definite NOEC were derivable. This would not represent the properties of Bisphenol A and therefore underestimate the effects, as the studies with effects even at the lowest test concentration are reliable. Using the lowest effect concentrations from a valid test would result in a LOEC of 0.000372 mg/L for *Danio rerio* based on mean-measured concentrations (or a NOEC < 0.000372 mg/L) and 0.000106 mg/L for *Marisa cornuarieties* based on median measured concentrations. ECHA Guidance R.10 specifies in table R.10-1 how to derive a NOEC from a LOEC if the effect is between 10 and 20 % (NOEC can be calculated as LOEC/2.). As there was more than 20 % effect in the *Danio*-study (35 %), this was no possibility for NOEC derivation. As described in chapter 11.5.2 it would also be possible to use the EC₁₀ 0.000038 mg/L (meas TWA) derived from a statistical reevaluation by Ratte (2009) instead of the LOEC. Both above mentioned values are above 0.0001 mg/L and below 0.001 mg/L. Therefore, for rapidly degradable substances a classification of Aquatic Chronic 1 with a M-factor of 10 applies.

Similar to the approach used for the acute aquatic hazard, for the long-term aquatic hazard a probabilistic approach (SSD) is shown below. According to ECHA Guidance R.10, for a reliable SSD especially the comparability of test conditions and different endpoints for long-term toxicity as well as specific modes of action and the differences between taxa need to be considered. The SSD model assumes a normal distribution of species sensitivities. The resulting HC₅ is 0.00805 mg/L, using the effect values in bold see Table 11. Note: For some species there are studies where no NOEC is available as there were effects even at the lowest concentration tested. In this case, when the use of the other NOECs available for the species would not cover the whole “picture”/properties of Bisphenol A, the LOEC is used and the HC₅ means a optimistic hazard concentration. According to ECHA Guidance R.10 NOEC values below the 5 % of the SSD (HC₅) need to be discussed, as this could be an indication that a particular sensitive group exists, implying that some of the underlying assumptions for applying the statistical extrapolation method may not be met. This is the case for *D. rerio* and *M. cornuarieties* (LOECs= 0.000372 and 0.000106 mg/L, respectively).

Therefore, for classification the lowest valid effect concentration (LOEC- see above) is used, resulting in a classification of Aquatic Chronic 1 with an M-factor of 10.



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“Goodness of fit” for the SSD acute EC₅₀ values for all taxonomic groups were calculated with the *E7X* 2.1 software (Van Vlaardingen, 2014).

Anderson-Darling test for normality				
Sign. level	Critical	Normal?		
0.1	0.631	Accepted		
0.05	0.752	Accepted	AD Statistic:	0.46289058
0.025	0.873	Accepted	n:	30
0.01	1.035	Accepted		

Kolmogorov-Smirnov test for normality				
Sign. level	Critical	Normal?		
0.1	0.819	Rejected		
0.05	0.895	Accepted	KS Statistic:	0.87261095
0.025	0.995	Accepted	n:	30
0.01	1.035	Accepted		

Cramer von Mises test for normality				
Sign. level	Critical	Normal?		
0.1	0.104	Accepted		
0.05	0.126	Accepted	CM Statistic:	0.08106453
0.025	0.148	Accepted	n:	30
0.01	0.179	Accepted		

A HC₅ of 0.00805 mg/L was obtained with lower and upper limits of 0.00017 and 0.00253 mg/L, respectively.

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Table 13: Comparison with criteria for long-term aquatic hazards

	Criteria for environmental hazards	Bisphenol A	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)	hydrolysis negligible >78.2 % after 28 days (O ₂ consumption) => readily biodegradable	Rapidly degradable
Bioaccumulation	Log Kow ≥ 4 BCF ≥ 500	Log Kow = 3.4 BCF ≤ 144	Not bioaccumulative (low potential for bioconcentration)
Aquatic Toxicity	Rapidly degradable substances: Cat. 1: NOEC ≤ 0.01 mg/L Cat. 2: NOEC ≤ 0.1 mg/L Cat. 3: NOEC ≤ 1 mg/L	Fish: 300d-LOEC= 0.000372 mg/L (mean measured) (<i>Danio rerio</i>) Invertebrates: 150d LOEC= 0.00025 mg/L (nominal; median-measured: 0.000106 mg/L) (<i>Marisa cornuarietis</i>) Algae: 96h-E _r C ₁₀ = 0.40 mg/L (n.a.) (<i>Skeletonema costatum</i>)	Aquatic Chronic 1, M= 10 <i>(based on fish-LOEC and invertebrate-LOEC)</i>

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

The most protective valid short-term toxicity concentration resulting in fifty percent effect is the 48h-LC₅₀ of 0.885 mg/L (measured) for *Acartia clausi*. This results in a classification of Bisphenol A as Aquatic Acute 1; H400 (M-factor of 1).

Bisphenol A is rapidly degradable and has a low acutal bioaccumulation. The most protective valid long-term toxicity no (in this case: lowest) observed effect concentration is 0.000372 mg/L (mean measured) for *Danio rerio*. This is supported by a further valid lowest observed effect concentration for *Marisa cornuarietis* of 0.000106 mg/L (median-measured). A determination of NOECs were not feasible, the LOECs represent a “best case”. This results in a classification of Bisphenol A as Aquatic Chronic 1; H410 (M-factor of 10).

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter’s proposal

Bisphenol A was classified under the Dangerous Substance Directive (DSD, Directive 67/548/EEC) for environmental effects with R52 ('Harmful to aquatic organisms', 30th ATP to DSD; Commission Directive 2008/58/EC). Currently there is no harmonised classification under the CLP Regulation for environmental hazards.

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The Dossier Submitter (DS) proposed to classify the substance as Aquatic Acute 1 - H400 (M=1) based on a 48-h mean measured EC₅₀ value of 0.885 mg/L for the marine crustacean *Acartia clausi*, and as Aquatic Chronic 1 - H410 (M=10) based on rapid degradation and a 300-d mean measured LOEC value of 0.000372 mg/L for the fish *Danio rerio* supported by a 150-d LOEC value of 0.000106 mg/L (median-measured)/0.00025 mg/L (nominal) for the snail *Marisa cornuarietis*. The determination of No Observed Effect Concentrations (NOEC) in the snail study was not feasible, therefore the Lowest Observed Effect Concentrations (LOEC) were used as an alternative. There are also other studies in the CLP report available which provide toxicities within the same range.

Bisphenol A has gone also through an EU risk assessment (European Commission, 2010). It has also been identified as a substance of very high concern based on its potential endocrine disrupting properties for both human health and environment (ECHA, 2017b). The proposed endocrine mode of action of the Bisphenol A requires additional consideration where the interpretation of the results obtained from the experimental studies in the CLH report is concerned.

Degradation

The physical and chemical properties of Bisphenol A suggest that hydrolysis under environmental relevant conditions is negligible.

Three OECD TG 301F tests (Manometric respirometry test) are available. In the first test using non-adapted inoculum from activated sludge from a municipal wastewater treatment plant, 78.2 to 81.0 % degradation based on O₂ consumption and 76.3 to 81.2 % degradation based on CO₂ production was indicated at day 28. In the second test using non-adapted inoculum derived from activated sludge from a municipal wastewater treatment plant, the O₂ consumption was found to be 85 to 93% after 28 days. A third test in which the activated sludge from a municipal wastewater treatment plant was used (adaptation not specified), reported 87.8±6.9 % O₂ consumption after 28 days. In all these tests, the 10-day window was fulfilled.

No degradation was observed in OECD TG 301D (Closed Bottle Test; 0 % O₂ consumption after 28 days), OECD TG 301B (Modified Sturm Test; 1-2 % CO₂ evolution after 28 days) and OECD TG 301C (0 % degradation after 14 days) tests. Bisphenol A was not inhibitory to micro-organisms under the test conditions in OECD TG 301D and OECD TG 301B tests.

All six studies are equivalent based on their reliability scoring (reliable with restriction; the deviations are mentioned in the CLH report). The DS also referred to additional studies on ECHA's dissemination page by the REACH Registrants but these studies were not used for classification of the substance by DS due to a lack of information on experimental details. Based on the available results, the DS concluded that Bisphenol A is readily biodegradable.

The degradation of Bisphenol A was examined in surface water by Klečka *et al.* (2001). The water samples from seven different rivers across the United States and Europe were collected upstream and downstream from wastewater treatment plants known to treat wastewater containing Bisphenol A. Two different methods were conducted: River-die-away studies for ¹⁴C-Bisphenol A (initial Bisphenol A concentrations 50-5500 µg/L) and respirometry studies (initial Bisphenol A concentration 5000 µg/L). Bisphenol A was not detected in the river samples prior to the addition of the test compound. Negligible losses of Bisphenol A were observed in autoclaved controls, indicating no abiotic degradation. There was no significant difference

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between the tests conducted with different river waters or river waters upstream or downstream from wastewater treatment plants. The results indicated rapid biodegradation of Bisphenol A after an initial lag phase.

In the ^{14}C river die-away studies lag periods of 2-8 days were observed and half-lives between 0.5 and 1.4 days were estimated. Degradation of ^{14}C - Bisphenol A resulted in mineralization with an average yield of 65-80 % $^{14}\text{CO}_2$ at the end of the test period (18 days). In the respirometry studies, after a lag period of 2.3-4.4 days, 59 - 96 % ThCO_2 was formed after 18 days. The estimated half-lives ranged from 0.5 to 2.6 days. In addition, the authors conducted studies with lower Bisphenol A concentrations (0.05 and 0.5 $\mu\text{g/L}$). Due to analytical limitations, only primary biodegradation was measured. After 28 days the Bisphenol A concentration was below 0.005 $\mu\text{g/L}$ and the estimated half-lives ranged from 3 to 6 days.

Two studies on primary degradation of Bisphenol A are available. In the first study, the primary degradation of Bisphenol A in river water was studied (Kang and Kondo, 2002). Under aerobic conditions Bisphenol A was rapidly primarily degraded with half-lives of 2-3 days. After 10 days the concentration was below the LOD. In the second study performed by same authors (Kang and Kondo, 2005), the primary degradation in seawater and in river water at different temperatures (25 °C, and 35 °C and additional 4 °C for seawater) was investigated. In river water, half-lives were 4 and 3 days at 25 °C and 35 °C, respectively. In seawater, lag periods of 30 days (25 °C and 35 °C) and 40 days (4 °C), respectively, were observed. At the end of the experiment (60 days), the initial concentration decreased to ~200 $\mu\text{g/L}$ at 25 °C/35 °C (80 % primary degradation) and ~700 $\mu\text{g/L}$ at 4 °C (30 % primary degradation). In autoclaved seawater, no degradation was observed over 60 days, indicating no abiotic removal process.

Ike *et al.* (2000) studied the degradation of Bisphenol A in 44 water microcosms. The river water microcosms were prepared from water samples from seven rivers, at 15 sites, with conditions ranging from clean to heavily polluted. Degradation was noted in forty of the river water systems. Six of the river water systems were able to mineralise the substance completely, and 34 others showed TOC removal of 40-90 %. Degradation tended to be greater in microcosms from more polluted waters. Two metabolites were identified: 2,3-bis(4-hydroxyphenyl)1,2-propanediol and p-hydroxyphenacyl alcohol. According to the DS, these metabolites cannot be removed by Bisphenol A-degrading bacteria.

The DS refers to the presence of additional studies on the degradation of Bisphenol A in surface water on ECHA's dissemination page (REACH Registration dossier). All studies showed rapid (primary) degradation of Bisphenol A in surface water.

Based on the available results and a weight of evidence approach, the DS concluded that Bisphenol A should be considered as **rapidly degradable** for classification purposes.

Bioaccumulation

The experimentally derived Log K_{ow} of Bisphenol A is 3.4 at 21.5°C and pH 6.4 (OECD TG 107).

Three bioaccumulation studies are available for Bisphenol A performed with fish and freshwater clams. In the first study performed according to the MITI guideline, carp (*Cyprinus carpio*) were exposed to Bisphenol A concentrations of 150 $\mu\text{g/L}$ and 15 $\mu\text{g/L}$ in a flow-through system for 6 weeks. The bioconcentration factors of <20 to 67.7 for low exposure and 5.1 to 13.3 for high exposure were measured.

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In the second study performed with freshwater clams (*Pisidium amnicum*), the uptake and depuration rates were measured using ^{14}C radiolabelled Bisphenol A at temperatures between 2 and 12 °C. Both uptake and depuration rates increased with temperature, although the uptake rate decreased slightly at the highest temperature. The bioconcentration factor was calculated from the concentration ratios at steady state and from the two rates. The maximum value was obtained at 8 °C by both methods as 144, based on concentrations and 134, based on rates.

In the third study, killfish (*Oryzias latipes*) were exposed to Bisphenol A concentration of 17 µg/L in a flow-through system for 6 days. Fish were analysed at intervals, and the results at 5 and 6 days showed that steady state had been reached. The mean BCF from these two times was 73.4 L/kg.

The DS concluded that Bisphenol A has a low potential to bioconcentrate and is therefore **not** considered a **bioaccumulative** substance for classification purposes.

Aquatic toxicity

The available database for Bisphenol A is large. The CLH report presents data from aquatic toxicity tests with 24 (acute) and 32 (chronic) species. For Bisphenol A, there are reliable aquatic acute and chronic toxicity data for fish, invertebrates, algae, aquatic plants and amphibians. All studies used for evaluation are rated with reliability index (R.I., Klimisch) score of 1 or 2 by the DS. All studies included were considered valid by the DS.

For determination of environmental hazard (aquatic toxicity reference value) the DS used two approaches, deterministic (using effect values from relevant and reliable experimental studies) and probabilistic (Species Sensitivity Distribution - SSD) approach. The probabilistic approach was used by the DS as supportive evidence for both acute and chronic hazard classifications.

Acute toxicity

The summary of the relevant information on acute toxicity is provided in Table 10 of the CLH report.

Deterministic approach

For fish, eight studies with five different fish species were available in the CLH dossier. Fathead minnow *Pimephales promelas* was the most sensitive fish species tested in the acute studies performed according to ASTM E-729-80, with a nominal 96 h LC₅₀ of 4.6 mg/L.

Twenty one studies with different taxonomic groups were provided for aquatic invertebrates. The geometric mean of 9.88 mg/L based on nine toxicity values for *Daphnia magna* (re-calculated after the consultation) was used as a representative toxicity value for this species. The lowest study value, according to ISO 14669:1999, resulted in a 48 h LC₅₀ (survival) of 0.885 mg/L (mean measured) for marine copepod *Acartia clausi* (Tato *et. al.*, 2018). Two other toxicity studies performed with aquatic invertebrates were reported by the DS, with toxicity values below 1 mg/L. In the first study (Andersen *et al.*, 1999), the 72 h EC₅₀ based on immobilization was 0.96 mg/L, while in the second study (Özlem and Hatice, 2008) a 72 h EC₅₀ value of 0.71 mg/L based on embryotoxicity was reported.

Five acute toxicity studies were available for algae and aquatic plants. The marine diatom *Naviculla incerta* was the most sensitive species tested in the acute studies performed

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according to similar to OECD TG 201, with a measured 96 h E_rC_{50} of 3.73 mg/L.

For other aquatic organisms (amphibians), there were non-guideline studies on African clawed frog (*Xenopus laevis*) and Argentine Toad (*Rhinella arenarum*) available with nominal 168 h LC_{50} of 7.1 mg/L and 72 h LC_{50} of 4.8 mg/L, respectively.

Species Sensitivity Distribution (SSD) approach (probabilistic approach)

In the CLH report, the DS pointed out that according to the CLP and REACH Guidance on information requirements and chemical safety assessment (Chapter R.10), in case of very large data sets, statistical techniques (e.g. HC_5 derivation via a SSD) can be used to estimate the aquatic toxicity reference value for classification (equivalent to using the lowest EC_{50}), when the criteria for applying the SSD approach are met. In the opinion of the DS, sufficient species (>10) and taxonomic groups (>8) are available to meet the criteria for applying the SSD approach. The lowest, reliable endpoints for a species or genera were used in the SSD. The acute freshwater and marine ecotoxicity values displayed in bold in the Table 10 of the CLH report were used for the SSD analysis. For *Daphnia magna*, a geometric mean of 9.88 mg/L calculated from 9 studies was used for the 48 h EC_{50} (re-calculated after the consultation).

The SSD model assumes normal distribution of species sensitivities. In the view of the DS, this may be assumed for acute toxicity as the endocrine mode of action of Bisphenol A rather exerts effects over longer periods of time (e.g. in long-term toxicity tests). The data followed a normal distribution according to three goodness-of-fit tests (Kolmogorov-Smirnov, Anderson-Darling and Cramer-von Mises). An HC_5 of 0.60 mg/L was obtained with lower and upper limits of 0.29 and 1.01 mg/L, respectively.

DS conclusion on acute aquatic classification

The acute aquatic classification proposed by the DS (based on the deterministic approach) was based on the Tato *et al.*, 2018 toxicity study with the marine copepod *Acartia clausi*. The DS proposed Aquatic Acute 1, with an M-factor of 1 based on a 48 h LC_{50} = 0.885 mg/L (mean measured). A very similar HC_5 value of 0.60 mg/L was obtained with the SSD approach. Both values are below 1 mg/L and support a classification of Bisphenol A as Aquatic Acute 1 with an M-factor of 1, in the proposal of the DS.

Chronic toxicity

The summary of the relevant information on chronic toxicity is provided in Table 11 of CLP report.

Deterministic approach

For fish, nineteen studies with nine different fish species are available in the CLP report. Zebrafish *Danio rerio* was the most sensitive fish species tested in the chronic studies with a mean measured 300 d LOEC of 0.000372 mg/L and mean measured 300 d NOEC of < 0.000372 mg/L based on effects on sex ratio, larval malformations and larval mortality (Chen *et al.*, 2015). There were two other studies with very low effect concentrations conducted with *Dania rerio*, a 5 m NOEC for reduced egg production of 0.000174 mg/L (mean measured) (Chen *et al.*, 2017) and NOEC for growth of < 0.01 mg/L (nominal) (Keiter *et al.*, 2012).

Twenty six studies with different taxonomic groups were provided for aquatic invertebrates. The geometric mean 3.23 mg/L based on four toxicity values for *Daphnia magna* was used as a representative toxicity value for this species. Marcial *et al.* (2003) determined a 21 d NOEC

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for developmental delay for marine copepod *Tigriopus japonicus* of 0.001 mg/L (nominal).

The gastropod *Marisa cornuarietis* was the most sensitive species tested. The study resulted in 150 d LOEC (effects on egg, clutch production) of 0.00025 mg/L (nominal) and 0.000106 (median-measured) (Oehlmann *et al.*, 2006). In this study, the NOEC could not be established because the lowest test concentration already exhibited significant effects. There is one another gastropod study with *Potamopyrgus antipodarum* by Sieratowicz *et al.* (2011) which resulted in a NOEC (increased embryo production) of 0.0046 mg/L (mean measured).

Three chronic toxicity studies were available for algae and aquatic plants. The marine algae *Skeletonema costatum* was the most sensitive species tested in the chronic studies performed according to EPA-560/6-82-002, with a measured 96 h E_bC₁₀ of 0.40 mg/L.

For other aquatic organisms (amphibian) there were four non-guideline studies on African clawed frog (*Xenopus laevis*) available with nominal NOEC values in range from 0.0073 mg/L to 0.5 mg/L, depending on test design and endpoint.

Species Sensitivity Distribution (SSD) approach (probabilistic approach)

In the CLH report, the DS pointed out that according to the REACH Guidance on information requirements and chemical safety assessment (Chapter R.10), in order for a reliable SSD approached to be used, the comparability of test conditions and different endpoints for long-term toxicity, as well as the specific modes of action and the differences between taxa need to be considered. The SSD model assumes a normal distribution of species sensitivities. The chronic ecotoxicity values displayed in bold in Table 11 of the CLP report were used for the SSD analysis. The DS indicated that for some species there were studies where no definite NOEC could be determined as there were effects even at the lowest concentration tested. In the view of the DS, it would not be appropriate to take into account only the studies in which these definite NOEC were able to be derived as this would not correctly represent the properties of Bisphenol A and, therefore, underestimate the effects. Therefore, when the use of the other NOECs available for the species would not adequately represent the properties of Bisphenol A, the LOEC was used. The HC₅ of 0.00805 mg/L was obtained with lower and upper limits of 0.00017 and 0.00253 mg/L, respectively.

DS conclusion on chronic aquatic classification

The DS proposed a chronic classification as Aquatic Chronic 1, with an M-factor of 10 based on the Chen *et al.* (2015) toxicity study using zebrafish (*Danio rerio*) that derived a 300 d LOEC of 0.000372 mg/L, based on mean-measured concentrations. The substance was considered to be rapidly degradable. After the consultation in the ECHA website, the view of the DS was that a 5m NOEC of 0.000174 mg/L (mean measured) from Chen *et al.* (2017) should be considered as the lowest chronic value for fish. These results are supported by a 150 d LOEC of 0.000106 mg/L for the gastropod *Marisa cornuarietis*, based on median measured concentrations. In this study, as determination of NOECs was not feasible, the LOECs represented, according to the DS, a "best case".

Comments received during consultation

Three Member States (MS) and one industry association submitted comments on the DS's proposal during the consultation. All commenting MSs agreed with the proposed aquatic acute classification as Aquatic Acute 1, with an M-factor of 1. Two commenting MSs supported aquatic chronic classification of the substance as Aquatic Chronic 1, with an M-factor of 10.

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The industry association disagreed with the DS proposal to classify the substance as Aquatic Acute 1, M-factor = 1 and Aquatic Chronic 1, M-factor = 10 due to, in their opinion, inappropriate application of the CLP criteria and inadequate study reliability rating by the DS. In their opinion, a more appropriate application of the CLP criteria and more adequate study reliability ratings would lead to a classification of Bisphenol A as no classification for aquatic acute toxicity and Aquatic Chronic 2. The industry association also performed their own statistical analyses that derived an HC₅ value of 0.0136 mg/L that would also support the classification of the substance in category Aquatic Chronic 2 (SSD argumentation are presented in this RCOM below). The following sections present a summary of the key issues raised by the industry association and MS, with the full comments, as well as the analytical DS and RAC responses, found in the RCOM document and later on in the current opinion document:

Taxonomic groups considered

The industry association pointed out that the classification proposed in the CLH report partly builds upon taxonomic groups other than fish, crustacea and algae/higher plants. Using test species that represent other trophic levels or are not typically required in a regulatory framework (e.g., snails, insects, amphibians) in the CLP process would be, in their opinion, contrary to the basic principle that the hazard of different substances should be compared on the same basis (i.e. same trophic levels/species when available). Therefore, the classifications for acute and chronic aquatic toxicity as proposed in the CLH report are not justified, in the commenter's opinion.

The DS responded that they followed the CLP guidance (version 5.0, July 2017, section 4.1.3.2.3.1, p.496) where it is stated that normally fish, crustacea and algae species is used to determine the toxicity of a substance **but** data on other species can also be considered under given conditions. Additionally, the CLP guidance (section 4.1.3.2.4.1) states that the three mentioned taxa are only a base/ minimum dataset that may be, in cases, a poor surrogate for the wide range of species in the environment and that a weight-of-evidence approach may be used for setting a classification (section 4.1.2.1.). RAC agrees with the DS as this is in line with the CLP regulation and guidance.

Reliability 1 studies only used

The industry association mentioned that the CLP Regulation requires that only data of high reliability are to be used for classification purposes. The reliabilities should normally be given by the criteria proposed by Klimisch et al. (1997) but additional criteria, related to specific substance properties, can often be helpful to underpin the generic assessment. Furthermore, the association noted that the proposed classifications are not based on fully reliable studies (Klimisch 1), but on studies which are rated reliable with restrictions (Klimisch 2) or should even be rated not reliable (Klimisch 3) – contrary to respective CLP guidance, which gives preference to fully reliable (Klimisch 1) studies for data rich substances, as is Bisphenol A. Several Annexes have been provided were the reliability of each experimental study in the CLH report has been re-assessed.

The DS agreed that only reliable and relevant studies should be used for CLP classification. Thus, studies fulfilling the Klimisch 1 **and** 2 criteria according to Klimisch *et al.* (1997) should be used and highlighted that this principle was followed in the CLH report. In addition, section 4.1.3.2.4.3. of the CLP guidance further states that the best quality data should be used and test conditions be clearly and completely articulated. The DS also agreed with the comment in

that the evaluation of study reliability can **also** be done according to other systematic approaches, e.g. CRED. RAC agrees with the DS as this is compatible with the CLP Regulation and Guidance requirements, for example section 4.1.3.2.1.

Use of Species Sensitivity Distributions (SSD) techniques

The industry association highlighted that no reference is given for the use of statistical extrapolation techniques and use of data on other taxonomic groups in weight-of-evidence methodology. This complex situation precludes the use of statistical extrapolation techniques for derivation of an acute hazard category for Bisphenol A because only a small set of validated OECD TGs is available, limited to key species of three trophic levels (fish, crustacea, algae) and that the basic paradigm of using statistical approaches in a regulatory context is to mirror effects on complex aquatic ecosystems by including toxicity data for test species of major taxonomic groups. Consequently, an acute hazard category should be derived using the conventional deterministic approach, i.e. selecting the lowest, fully valid LC/EC₅₀ value for fish, crustaceans, and algae. In case of chronic classification, the view of the industry association was in line with the approach taken by DS. The statistical extrapolation techniques, using valid NOECs from a broad range of aquatic taxa, should be used as supportive evidence for derivation of a chronic hazard category.

Concerning the use of the SSD approach, the DS pointed out that in the CLP Guidance (p. 502), referring to the REACH Guidance on information requirements and chemical safety assessment (Chapter R.10), the use of the Species Sensitivity Distribution approach is described as a possibility for very large data sets, meeting certain criteria for applying it. The CLP guidance (section 4.1.3.2.4.3) does not distinguish between short- and long-term data for the applicability of the SSD approach. In the view of the DS, for data-rich Bisphenol A the REACH guidance considerations are fulfilled and are presented in the CLH report. Based on the comments received during the consultation in the ECHA website, the DS revised the data used for the derivation of HC₅ that resulted in a value of 0.000543 mg/L.

Based on the considerations presented in Supplemental information - In depth analyses by RAC/ background document (BD), RAC is of the opinion that the SSD approach for acute and chronic classification should be used as additional information. The acute and chronic toxicity dataset used for the SSD approach and calculation by RAC are also presented therein.

Individual acute and chronic toxicity study reliability

In the view of the industry association the dataset in the CLH report on acute and chronic aquatic toxicity consists of a selection of studies which is partly based on inappropriate reliability ratings of scientific studies by the DS. Specifically, in their view it comprises various studies that do not meet the minimum requirements for scientific reliability and are, therefore, not adequate for classification purposes. In brief, the industry association claimed major deficiencies in study design, methodologies and weak documentation as reasons for a downgrade in the Reliability scoring. The DS generally disagreed with the industry commentator's evaluation repeating that the evaluation took place following the Klimisch and CRED principles. Based on the comments received, the DS did not change the opinion regarding reliability of the studies. More information on the study quality is provided in the BD.

Specific comments from individual MSs

One MS commented on the following issues:

Acute classification: The MS asked whether the key aquatic acute toxicity study performed with

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the marine crustacean *Acartia clausi* (Tato *et al.*, 2018) was considered in the dossier or assessment of triclosan and 4-nonylphenol. The DS responded that the study was not used in the assessment of the above-mentioned substances. The same MS was of the opinion that the key aquatic acute toxicity study (Tato *et al.*, 2018) should be considered as Klimisch 2 and not Klimisch 1 because the study was not conducted to GLP and some study details were missing (lack of raw data, incomplete dissolved oxygen data, no information on the culture history, incomplete information on final solvent concentrations and full details of reference compound studies). The DS provided additional data on the biological quality of the stock and that the study was run according to ISO guideline 14669:1999. In the view of RAC, the study by Tato *et al.* (2018) is reliable and should be used as a key study for setting the hazard classification.

Chronic classification: In view of the MS the aquatic chronic toxicity studies performed with fish *Danio rerio* (Chen *et al.*, 2015; Chen *et al.*, 2017) were not valid for hazard classification due to several deficiencies.

For the Chen *et al.* (2015) study, different deficiencies were indicated by the MS. The DS agreed that study was not run according to GLP and that the study result would underestimate the effect with a missing no effect concentration. However, the DS pointed out that many of the test conditions in the study were similar to the ones recommended in OECD TG 234, it provided information on replicates and their variability for endpoint sex ratio and considered the study valid and reliable. RAC agrees with the DS.

Similarly, the MS mentioned several deficiencies regarding the Chen *et al.* (2017) study. The DS responded by providing more study details and still argued that the study is valid and reliable. RAC agrees with the DS.

The MS also questioned if the LOEC value from the aquatic chronic toxicity study with gastropod *Marisa cornuarietis* (Oehlmann *et al.*, 2006) was sufficiently reliable to be considered as the key chronic classification endpoint or whether the study endpoint should be considered supporting information (detailed assessment of the study was provided in the RCOM). The commenting MS pointed out that other, more reliable, studies with gastropod *Marisa cornuarietis* did not replicate the same level of effects although there were differences with study design and test species. The DS responded that the effects endpoint egg/clutch production supported the results from studies with fish and showed that sensitive organism groups are indeed adversely affected in this concentration range. Regarding the other studies with the gastropod *Marisa cornuarietis*, the DS pointed out that these studies were very different with respect to study design, test conditions and test species/strain, explaining in that way the higher effects levels of Warbritton/Forbes study that should be seen as different and not comparable studies.

Regarding the aquatic chronic toxicity study performed with *Salmo trutta* (Lahnsteiner *et al.*, 2005), the same commenting MS was of the opinion that due to limitations and uncertainties this study is not robust enough to be used in the SSD dataset. In the view of the DS, the cited limitations do not devalue the study and only for the endpoint 'time point of ovulation', for which only 6 fishes were used, is the test not robust enough and the related results were not taken into account. For the endpoints egg production and semen fertility, the study was evaluated as Klimisch 2. In the view of RAC, as explained later on in the opinion, the study is not reliable and should not be used for hazard classification.

Regarding the SSD approach, the MS asked for clarification regarding the use of values for different species, endpoints and inclusion or exclusion of algae and aquatic plants in SSDs. The DS clarified that the most sensitive reliable endpoints for a species or genera have been used

and that the influence of inclusion/exclusion of values has been checked (details provided in RCOM document). The DS also provided the SSD calculations with and without algae and aquatic plants to show any differences.

The second commenting MS provided comments regarding the lack of a detailed description of the studies in the CLH report that were used in the SSD (e.g. purity, test regime, concentrations maintained, etc.). In addition, the MS requested clarification on the use of some study results in the SSD. The MS highlighted that nine reliable aquatic acute toxicity studies with *Daphnia magna* were available in the CLH report and all of them should be used for the calculation of a geometric mean. The DS agreed and recalculated the geometric mean which resulted in an EC₅₀ of 9.88 mg/L (instead of 9.47 mg/L).

A third MS also asked for an explanation as to why the NOEC of 0.00017 mg/L for *Danio rerio* (Chen *et al.*, 2017) was not considered the lowest chronic value for fish by the DS instead of the LOEC of 0.000372 mg/L (Chen *et al.*, 2015). The DS agreed that the NOEC from Chen *et al.* (2017) should be considered as the lowest chronic value for fish as this is a "real" NOEC which was not derivable from Chen *et al.* (2015).

Regarding ready biodegradability of Bisphenol A, the same MS pointed out that no explanation was given for the divergence of equally reliable results. The DS explained that the evaluation of degradation of Bisphenol A was based on a weight of evidence approach including studies of an equivalent reliability. In this context, the studies with positive results (OECD TG 301F) were of good scientific quality and the test conditions were well documented.

In the view of this MS, the inoculum used in the simulation study (Klečka *et al.*, 2001) may be adapted to Bisphenol A due to the sampling location near to a wastewater treatment plant treating Bisphenol A, which could lead to an improved biodegradation capacity. The DS agreed and explained that the half-life in this study is in the same order of magnitude of the half-lives from the studies investigating primary degradation.

The MS pointed out that in other freshwater studies only primary degradation was reported. Although half-lives are <16 days, no information is given on the classification of the degradation products. The DS responded that no degradation products were identified in the studies on primary degradation. Only the study from Ike *et al.* (2000) identified any metabolites.

Assessment and comparison with the classification criteria

Degradation

Bisphenol A is hydrolytically stable under environmentally relevant conditions. There are six valid and equally reliable ready biodegradation studies available, three of which showing that the substance is readily biodegradable (OECD TG 301F) and three not (OECD TG 301D, OECD TG 301B and OECD TG 301C). In line with the current CLP Guidance (version 5.0, July 2017, section II.3.5, p. 569), RAC is of the opinion that Bisphenol A should be considered readily biodegradable following the three valid OECD TG 301F studies and the 10-day window criteria being fulfilled, including the use of non-adapted inoculum.

In an aerobic water simulation study, whole system half-life for Bisphenol A was between 0.5 and 2.6 days at 20°C. Rapid primary degradation with half-lives between 2 and 4 days in water were reported in two other water/sediment simulation studies. However, information on transformation products is lacking (amount, identity, classification). Another water/sediment

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simulation study in which 40 – 100 % degradation (TOC removal) was observed identified degradation products (2,3-bis(4-hydroxyphenyl)1,2-propanediol and p-hydroxyphenacyl alcohol). Classification information for identified degradation products is lacking. Following the CLP guidance (version 5, July 2017, section II.3.4, p. 569) data on primary degradation may be used for demonstrating rapid degradability only when it can be satisfactorily demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment.

Bisphenol A is considered readily biodegradable following a valid OECD TG 301F study and the 10-day window criteria are fulfilled and, as the OECD TG 301F test is considered to be performed in stringent conditions, RAC is of the opinion that Bisphenol A should be considered as **rapidly degradable** for the purpose of aquatic hazard classification.

Bioaccumulation

RAC agrees with the DS that Bisphenol A has a **low potential to bioaccumulate** in aquatic organisms. The basis for this is that measured BCF values in aquatic organisms were below the decisive CLP Regulation criterion of 500 (BCF = 144). This is supported by the Log K_{ow} value being below the CLP Regulation criterion of 4 (Log K_{ow} = 3.4).

Aquatic acute toxicity

As reported before, the Species Sensitivity Distribution (SSD) approach (HC₅ value of 0.60 mg/L) for aquatic acute classification of Bisphenol A was considered as additional evidence by the DS. As elaborated in the BD, RAC acknowledges the merits of using the SSD approach for a data-rich substance such as Bisphenol A and has tried to replicate the related computations, resulting in a HC₅ value of 0.83 mg/L, which is in alignment with the DS calculations. However, RAC is of the opinion that in the presence of good quality experimental information for Bisphenol A, classification of the substance for acute aquatic hazard should primarily be based on a deterministic approach (most sensitive species) and that the SSD approach for acute classification should be used as additional information due to some of the uncertainties described in the BD.

According to the toxicity data presented in Table 10 of the CLP report, invertebrates are the most sensitive trophic level. Apart from the entire acute aquatic dataset, three studies deriving an EC₅₀/LC₅₀ below or equal to 1 mg/L have been comprehensively assessed by the RAC, with the detailed assessment presented in the BD. Based on this comprehensive analysis, all three studies (Tato *et al.*, 2018; Andersen *et al.*, 1999; Özlem and Hatice, 2008) were deemed reliable, albeit some drawbacks that in RAC's opinion do not have a serious impact on the study outcomes. Thus, RAC believes that data from these studies have been generated according to internationally accepted guidelines, appropriate both standard and "non standard" species were used, with *Acartia tonsa* being taxonomically the closest equivalent species to *Acartia clausi* (same genera).

RAC proposes to base the aquatic acute classification on the study performed with the marine copepod *Acartia clausi* with a 48 h mean measured EC₅₀ value of 0.885 mg/L. This result is supported by the two other studies that also provide toxicities within the same range (72 h EC₅₀ = 0.96 mg/L, Andersen *et al.*, 1999 and 72h-EC₅₀ = 0.71 mg/L, Özlem and Hatice, 2008). Based on these values Bisphenol A warrants classification as **Aquatic Acute 1, M-factor of 1**, as the acute toxicity is in the range $0.1 < L(E)C_{50} \leq 1$.

Aquatic chronic toxicity

RAC is of the opinion that in the presence of good quality experimental information for Bisphenol A, classification of the substance for chronic aquatic hazard should be based on a deterministic approach (numerical results from experimental studies and use of the most sensitive species) and that the SSD approach for chronic classification should be used as additional information due to some of the uncertainties presented in the BD. As reported before, the SSD approach (HC₅ value of 0.000543 mg/L) for aquatic chronic classification of Bisphenol A was considered as additional evidence by the DS. As elaborated in the BD, RAC acknowledges the merits of using the SSD approach for a data-rich substance such as Bisphenol A and has tried to replicate the related calculations, resulting in a HC₅ value of 0.0012 mg/L

According to the toxicity data presented in Table 11 of the CLP report, four studies with fish, one study with crustacea, three studies with mollusc and one study with amphibians showed NOEC/LOECs below 0.01 mg/L. All of these studies, as the most conservative ones, apart from the entire chronic aquatic dataset, have been assessed in depth by the RAC, as presented in the BD. Based on that comprehensive analysis, two studies for fish (Chen *et al.*, 2015; Chen *et al.*, 2017), one for crustacea (Marcial *et al.*, 2003) and one for mollusc (Oehlmann *et al.*, 2006) have been found reliable and appropriate to use for classification purposes.

One toxicity test with fish (Lahnsteiner *et al.*, 2005) and one with amphibians (Levy *et al.*, 2004; Pickford *et al.*, 2003 & 2010) have been found not to be reliable by RAC. RAC considers the study by Lahnsteiner *et al.* (2005) not reliable due to the fact that fish derived from a wild population (caught by electroshocking) were used, no information on the health of the fish and possible influence of environmental factors was available, issues with the acclimatisation of the test animals, small numbers of fish were used, no replicates, low statistical power based on low number of individuals at each endpoint and no analytical confirmation.

In the view of RAC the amphibians study as cited by Levy *et al.* (2004) should not be considered for classification due to the absence of a concentration-response relationship, insufficient replication, statistical methodology introducing statistical bias and the absence of significant incidences of gonadal abnormalities at the histological level.

RAC is also of the opinion that the NOEC of 0.0046 mg/L (mean measured) at 7 and 25 °C from the Sieratowicz *et al.* (2011) study is not reliable because the study was not carried out at the required temperature (16°C) in OECD TG 242. Consequently, the NOEC (0.0194 mg/L (mean measured)) at 16°C should be used for classification.

RAC considers the toxicity study with fish by Keiter *et al.* (2012) as additional information due to the presence of PFOS in the test water and miscounting of numbers of fish per aquaria.

From the four studies found reliable based on RAC's assessment, the Committee proposes to base the chronic classification on the study performed with fish *Danio rerio* with 5 m NOEC value of 0.000174 mg/L (mean measured) (Chen *et al.*, 2017). This result is supported by one fish (Chen *et al.*, 2015), one crustacea (Marcial *et al.*, 2003) and one mollusc (Oehlmann *et al.*, 2006) studies, which provide toxicities within the same range. Based on these toxicity studies and the substance considered as rapidly degradable, RAC agrees that Bisphenol A warrants classification as **Aquatic Chronic 1** with an **M-factor** of **10** (0.0001 < NOEC ≤ 0.001).

Supplemental information - In depth analyses by RAC

Considerations concerning the use of the species sensitivity distribution (SSD)

The Bisphenol A ecotoxicity dataset is a data-rich one, therefore, RAC is of the opinion that the SSD approach could in principle be applied for derivation of an HC₅ value. However, RAC considers that the available environmental dataset presented in the CLH report cause some uncertainty regarding its use in this specific case. Based on the available dataset, the following uncertainties are highlighted by the RAC:

- Among the studies which were comprehensively assessed by the RAC, some studies were found not reliable by RAC in contrast to the DS conclusion on their validity (e.g. Lahnsteiner *et al.*, 2005);
- Endocrine disruption mode of action that generated several different effect endpoints not uniformly identified in all studies and possibly suggesting different non-narcotic chemical sensitivities to different species. This, in turn, introduces uncertainties on the suitability of given taxa as to whether they can be included in the same SSD regression as other species;
- Some effect values used by the DS in the SSD computations were found not suitable or reliable by the RAC. For example, in the view of RAC the NOEC from the acute study (Özlem and Hatice, 2008) could not be used as a chronic value in the SSD, whilst RAC also disagrees with the DS regarding the use of the NOEC from the Sieratowicz *et al.*, 2011 (explanations provided later on);
- RAC has identified mistakes in the numerical values of some of the selected data by the DS. For example, for Kiyomoto *et al.* (2005) and Benstead (2010);
- RAC also acknowledges a comment during the consultation regarding the inclusion or not of results generated for algae and aquatic plants data and whether these species exhibit a lower sensitivity to Bisphenol A as a result of its endocrine mode of action;
- Lack of scientific consensus on the exact conditions for its use for short-term effects as exhibited by the wording of the respective REACH Guidance document.

Short-term toxicity study with copepod *Acartia clausi* (Tato *et al.*, 2018)

The study by Tato *et al.* (2018) is considered by the DS as Klimisch 1 while the opinion of the industry association and one commenting MS is that the study should be considered as Klimisch 2. The arguments are provided in the RCOM.

RAC acknowledges the shortcomings of the short-term toxicity study with copepod *Acartia clausi* (Tato *et al.*, 2018). However, RAC notes that

- The study was performed in accordance with an internationally accepted guideline.
- Deviations from standard guideline were made and are clearly described in the published article. In the view of RAC, the deviations do not have any impact on the study results.

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- *Acartia tonsa* is the taxonomically closest equivalent species to *Acartia clausi* (same genera), therefore the use of ISO guideline is suitable and thus the guideline validity criteria could be applied for *Acartia clausi*.
- In the ISO guideline¹, acetone is cited as example of an organic solvent of low toxicity to copepods which can be used in acute toxicity test. According to OECD TG document No. 23, the dimethyl sulfoxide (DMSO) could be used as a solvent in aquatic toxicity testing. Therefore, RAC is of the opinion that DMSO is an acceptable solvent. A published article states that controls run in all experiments were DMSO solutions in filtered sea water (FSW) at the highest concentration used in each test, normally 0.1% (v/v), always at values below NOEC for each test species (0.4 g/L for *A. clausi*).
- A requirement of the ISO guideline is that the density of copepods does not exceed 1 per 0.5 mL of solution. The density of 1 copepod/2 mL solution reported in the study is within the requirement.
- In the CLH report, the concentrations used are reported (150, 300, 600, 1200 and 4800 µg/L). The measured concentrations ranged from 96.2 to 106.7 % of nominal. In the published article it is stated that the Bisphenol A was very stable in seawater, showing less than 6% d⁻¹ reduction irrespective of light conditions.

Despite the shortcomings expressed above and in the RCOM, RAC considers the 48 h LC₅₀ (survival) of 0.885 mg/L (measured) for marine copepod *Acartia clausi* as relevant and reliable result therefore is appropriate to use the study as the key study for setting the hazard classification.

Short-term toxicity study with copepod *Acartia tonsa* (Andersen et al., 1999)

The study by Andersen *et al.* (1999) is considered by the DS as Klimisch 2 and relevant for classification, while the opinion of the industry association is that the study should be considered as Klimisch 3 (not reliable) because there is not sufficient information provided for a judgement of its reliability and the study shows severe shortcomings in the methodological details (provided in RCOM). Therefore, the results of the study are not appropriate for classification purposes, according to them.

The acute toxicity study by Andersen *et al.* (1999) is published as a scientific article that merely states that the acute toxicity of Bisphenol A to *A. tonsa* was detected according to ISO/DIS 14669 and the result was provided. Thus, RAC could not independently assess the reliability of the test. No additional information is available to RAC in Risk Assessment Report (European Commission, 2003, 2008 and 2010), confidential part of the CLP report and on ECHA's dissemination page by the REACH registrants.

The study was considered for derivation of the marine PNEC for Bisphenol A in derivation of proposed environmental quality standards for Bisphenol A². The test is done according to an

¹ <https://www.sis.se/api/document/preview/895679/>

² https://circabc.europa.eu/webdav/circabc/env/wfd/Library/working_groups/priority_substances/2a%20-%20Sub-Group%20on%20Review%20of%20Priority%20Substances%202014%20start/Substance%20dossiers%20-%20DRAFT/ Table%204.1%20Scoping%20Report %20substances%20-%20at%204%20September%202014/Bisphenol%20A%20Proposed%20EQS%20Report%20-%20Final%20Draft.pdf

ISO standard and no deviations from the standard guideline are described in the published scientific article. RAC notes that in the ISO guideline 14889:1999 it is stated that it may be appropriate for certain purposes to extend the exposure period to 96 h and to determine the 96 h LC₅₀. The 72 h EC₅₀ is not mentioned in the guideline. No justification is given in the publication for using 72 h EC₅₀. RAC considers the study reliable, but it is only used as supporting information for acute toxicity classification

Short-term toxicity study with *Paracentrotus lividus* (Özlem and Hatice, 2008)

The study by Özlem and Hatice (2008) is considered by the DS to be Klimisch 2 and relevant for classification while the opinion of the industry association is that the study should be considered as Klimisch 3 due to insufficient information in the published paper (e.g. no analytical confirmation of test concentrations, no information on: acclimation, feeding, sample size, replication, etc.), methodological deficiencies and unclear relevance of the reported endpoint. In view of the industry association, the results of the study are not appropriate for hazard classification under CLP.

RAC agrees with the DS that developmental defects of living pluteus larvae (embryotoxicity) after 72 h of exposure could be considered as an acute endpoint. The evaluated endpoint is immobilisation. RAC notes that there is an ASTM (E-1563 – 98, 2012)³: Standard Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos which is considered an acute toxicity test and the effects of the test material on development embryos and larvae are determined during 48 to 96 h.

RAC agrees that some information is missing (e.g. acclimatization, feeding, time of the year the study was performed, quality of the fertilized eggs, no analytical confirmation of concentrations tested) but the following is provided in the published article or by the DS in the RCOM document:

- The solvent concentration used is not specified but in the test dimethyl sulfoxide (DMSO) was used as a solvent control. In the published article, it is stated that the DMSO has been known as an environmentally friendly solvent and is not classified as hazardous. In addition, embryos were exposed to DMSO as a solvent control, being added to the medium in the same volume as the highest test concentration of Bisphenol A, and no effect was detected on the development of sea urchin embryos.
- Information regarding replication is available in the published article and by the DS in the RCOM document. All treatments were tested as six replicates.
- The sample size is provided by DS in the RCOM document. 100 embryos were observed.

In conclusion, despite the associated uncertainties RAC considers the 72h EC₅₀ (immobilization) of 0.71 mg/L based on nominal concentrations reliable, but it is only used as supporting information for acute toxicity classification.

It should be noted that in the same study, a 72 h NOEC based on larval malformations is available which was considered by the DS for chronic classification. This was criticised by the industry association during consultation, pointing out that an exposure duration of 72 hours, covering only a short developmental window, is not a chronic but an acute toxicity test and

³ <https://www.astm.org/Standards/E1563.htm#s00085>

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therefore should not be used for chronic classification. RAC agrees with industry association. In the CLP guidance (version 5.0, July 2017, section I.3.2, p. 558) it is stated that chronic toxicity, for purposes of classification, refers to the intrinsic property of a substance to cause adverse effects to aquatic organisms during exposures which are determined in relation to the life-cycle of the organism. RAC is of the opinion that 72 hours (larval stage) starting from 10 minutes after fertilization should not be regarded as a chronic exposure considering the life-cycle of sea urchin *Paracentrotus lividus*. Sea urchins reach the sexual maturation after 2 to 3 years depending on the habitat and food.

Long-term toxicity study with fish *Danio rerio* (Chen et al., 2015)

The DS considered the study as Klimisch 2. The reliability of the study endpoint and overall study quality was questioned during the consultation by one Member state and an industry association (refer to RCOM document). Based on the comments received during the consultation the DS disregarded the 300d-LOEC value of 0.000372 mg/L and considered the value from another fish chronic toxicity study (Chen et al., 2017) as the lowest chronic value for fish.

RAC acknowledges that the study has some shortcomings, nevertheless the study is considered reliable by RAC due to the following reasons:

The endpoint shift in sex ratio was determined visually and histologically. The sex ratio of the control fish was 57% females, which is within the validity criteria of OECD TG 234 (30 to 70% males or females). The variability is also provided in the RCOM.

RAC is aware that in the study only a solvent control was used. However according to OECD TG No. 23 the dimethyl sulfoxide (DMSO) was found to be effective for aquatic toxicity testing because it does not affect the outcome of the study. In addition, the concentration of the DMSO (0.01%) used in the study is within requirements of OECD TG No.23 and OECD TG 234 (should not be greater than 0.1 mg/L). Therefore, RAC is of the opinion that DMSO does not have any impact on the study results and the lack of negative control does not affect the reliability of the result.

The information about stock density/sample size, number of fish and replication is provided in the published article and CLP report.

RAC is of the opinion that the study is less reliable due to:

- There are some uncertainties with regards to the following conditions: The precise oxygen saturation was not measured. Variations in exposure tank temperature were reported (22°C in winter and 25°C in summer (personal communication of the registrant with the author)). OECD TG 234 recommends 27± 1.5 °C for zebrafish.
- The study has some drawbacks with respect to analytics. Bisphenol A was detected in the solvent control at a measured level of 0.000032 mg/L (that is more than eleven times lower than test concentration with effect).

The test was performed as a limit test with one concentration.

In conclusion, although there are some uncertainties regarding the test setup, RAC considers the endpoint shift in sex ratio from the Chen et al. (2015) study (NOEC of 0.000372 mg/L) a relevant and reliable endpoint for chronic hazard classification.

Long-term toxicity study with fish *Danio rerio* (Chen et al., 2017)

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The DS considered the study as Klimisch 2 and relevant for classification. The reliability of the study endpoint and overall study quality was questioned during the consultation by one Member state and the industry association (RCOM document).

RAC acknowledges that some information regarding experimental design is missing (e.g. source of fish, acclimatization). However RAC recognizes the following:

- Experiment starts with embryos obtained from adult fish, therefore lack of data for acclimatization would not devalue the study. The embryos were collected, pooled, and divided into three exposure groups. All groups were raised under identical conditions until the beginning exposure time for each group, at which point fish water was replaced with the Bisphenol A exposure solution.
- In the study only was the solvent control used. However, according to OECD TG No. 23 the acetone was found to be effective for aquatic toxicity testing because it does not affect the outcome of the study. In addition, the concentration of the acetone (0.001% (v/v)) used in the study is within requirements of OECD TG No.23 and OECD TG 234 (should not be greater than 0.1 mg/L). Therefore, RAC is of the opinion that acetone does not have any impact on the study results.
- The justification for exposure renewal is provided in the published article. In the article it is stated that previous studies have demonstrated that the half-life for Bisphenol A degradation in native lake water is 15 and 17 days for 5 and 50 ppm, respectively.
- RAC agrees that there are some uncertainties regarding the maintenance of test concentrations, especially from 3 to 6 mpf. Bisphenol A levels in the exposure solution without zebrafish is not significantly changed during the 14-day test period (experimental result). Bisphenol A is stable for 7 days in the presence of fish (experimental result). The only period with Bisphenol A concentrations may be lower than nominal is from 3 to 6 mpf.
- According to published article the water quality parameters were monitored on a weekly basis but no details or data are available to RAC.
- For analysis of the exposure to chemicals, the HPLC method with fluorescence detection was used which demonstrated an adequate linear fit. The method is assumed to be validated as required for a REACH registered substance. LOD not reported.
- Information regarding replicates are provided in the published article and in RCOM by DS and industry association. Three replicates were used in all three developmental stages.
- Information about number of fish per replicate is available in the published article and in RCOM by DS. The breeding trials (6 females x 6 males per replicate) x (3 replicates) within the same treatment groups were carried out to produce offspring for assessment of egg production.
- Segner *et al.* (2003) and Keiter *et al.*, 2012 are cited only in the introduction part of Chen *et al.* (2017) therefore no indication on corroboration could be seen by RAC.
- No clear dose response relationship was observed in this study.

In conclusion, RAC considers the 5 m NOEC of 0.000174 mg/L based on egg production for *Danio rerio* as a relevant and reliable result. Consequently, it is appropriate for us under CLP.

Long-term toxicity study with *Salmo trutta* (Lahnsteiner *et al.*, 2005)

The DS considered the study as Klimisch 2. The reliability of the study was questioned during

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consultation by one Member state and an industry commentator (refer to RCOM document).

The study by Lahnsteiner *et al.* (2005) is published as a scientific article and detailed information on experimental design and test conditions is missing. However, RAC acknowledges the following:

- In this study, the design of the flow through system used is very precise and RAC assumes that nominal concentrations could be close to real exposure concentrations
- Additionally, RAC is aware that in the study only the solvent control was used and that varying solvent concentrations were used. However, in the CLP report and as pointed out by the DS in RCOM, the dimethyl sulfoxide (DMSO) was used with concentrations following OECD TG No. 23. Therefore, RAC is of the opinion that DMSO does not have any impact on the study results. The industry association also agrees that solvent concentration is in an acceptable range. Based on this, RAC believes that the lack of negative control does not itself affect the reliability of the result.

However, RAC is also of the opinion that the study has the following deficiencies that put the reliability of the study into question:

- For the experiment, fish derived from a wild population (caught by electroshocking) were used and no information on the health of the fish and possible influence of environmental factors is available.
- Issues with the acclimatisation of the test animals
- Only small numbers of fish were used for control and test concentrations. In the study, four experimental groups were formed each consisting of 10 males and 6 females. No replicates were used.
- Low statistical power based on low number of individuals at each endpoint
- No analytical confirmation at least for initial concentration of Bisphenol A

Due to the deficiencies of the study presented above, RAC considers the study not appropriate for use under CLP.

Long-term toxicity study with *Tigriopus japonicus* (Marcial *et al.*, 2003)

The DS considered the study as Klimisch 2 while the opinion of the industry association is that the study should be considered as Klimisch 3 due to major deficiencies (e.g. lack of analytical confirmation of exposure concentrations and questionable statistical significance of observed effects).

In this study, no analyses of the test substance concentrations were performed. Nevertheless the test solutions were renewed daily by replacing around 50% of the working volume each time. Therefore, RAC assumes that nominal concentrations could be close to real one. For maturation stage the dose-response relationship was observed. Parents exposed to the highest test concentrations (0.001 and 0.01 mg/L) of Bisphenol A took significantly longer to mature than the control.

RAC considers the 21 d NOEC (developmental delay) of 0.001 mg/L (nominal) for marine copepod *Tigriopus japonicus* a relevant and reliable result. Therefore, it is appropriate for chronic classification.

Long-term toxicity study with mollusc *Marisa cornuarietis* (Oehlmann *et al.*, 2006)

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The DS considered the study as Klimisch 2 while the reliability of the study was questioned during the consultation by a commenting MS and industry association (refer to RCOM).

RAC acknowledges that there are some uncertainties regarding exposure concentrations. From the available information, it is evident that it is difficult to estimate measured concentrations due to a decrease in test concentrations between renewal cycles in the semi-static test design and due to difficulties in statistical analysis as pointed out in RCOM. Therefore RAC agrees with the DS that the median measured LOEC of 0.000106 mg/L based on egg and clutch production should be used for classification purposes.

RAC compared Oehlmann *et al.* (2006) and Forbes *et al.* (2007a and b, 2008) studies and found out that the studies could not be compared due to different experimental design and experimental conditions, which do not support the direct comparison of the results obtained. Oehlmann *et al.* (2006) observed that ramshorn snails *Marisa cornuarietis* exposed to Bisphenol A produced significantly more clutches and eggs. The effect depends on temperature and spawning period. These observations were not replicated in three studies of Forbes *et al.* (2007a, 2007b and 2008). It is evident that the most important parameters temperature and spawning period are different and results of Forbes *et al.* (2008) could not be directly compared with results of Oehlmann *et al.* (2006). Even more at relatively high temperature and during spawning the effect of increased rate of egg production is not seen in both studies due to the so called "masking effect". Additional investigations, Sieratowicz *et al.* (2011), performed for snail species *Potamopyrgus antipodarum* confirmed the strong temperature dependency of the response and endpoints for specie *Potamopyrgus antipodarum*. Temperature dependant NOECs were provided. The next study of Sieratowicz *et al.* (2011) for *Potamopyrgus antipodarum* supported observations in Oehlmann *et al.* (2006) for strong temperature influence on eggs and clutch production in snails.

During the consultation two confounding variables (snail density, presence of Bisphenol A in control) were pointed out that cast additional doubt on the findings of the study. RAC notes that the snail's density in Oehlmann *et al.* (2006) varied between 1.1-0.72 snails/L what is in line with OECD TG 243 (*Lymnea stagnalis* Reproduction Test) in which no more than 5 snails/L is recommended. Single positive determination of Bisphenol A in the control tanks was observed.

Overall, although there are some uncertainties in regards to exposure concentrations, RAC considers the 150 d LOEC of 0.000106 (median measured) based on egg and clutch production relevant and reliable and therefore is appropriate to use this study for chronic classification.

Long-term toxicity study with mollusc *Potamopyrgus antipodarum* (Sieratowicz *et al.*, 2011)

In the CLP report, the study by Sieratowicz *et al.* (2011) is considered well-documented, valid and meets the quality criteria of the TG as conducted during pre-validation of TG 243. The study is rated as Klimisch 2 by DS and industry association. However the reliability of the NOECs was questioned during the consultation. It was pointed out that only the NOEC at 16°C is accepted because the published OECD TG test method 242 (*Potamopyrgus antipodarum* Reproduction Test) requests to conduct the study at 16 °C as only this temperature is within the optimum temperature range of this species in the natural habitat.

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RAC agrees with the industry association that only the NOEC (0.0194 mg/L (mean measured)) at 16 °C should be used for classification because this is the required temperature in OECD TG 242.

Long-term toxicity with fish *Danio rerio* (Keiter et al., 2012)

The DS considered the study as Klimisch 2 while the reliability of the study was questioned during the consultation by an industry association (refer to RCOM). As pointed out during the consultation by an industry association, the study is well designed but there are some methodological deficiencies and irregularities that make it unsuitable for use in hazard assessment.

In the study, the Bisphenol A was not analytically verified. However, a flow-through system with a three-fold water exchange per day was applied throughout the study in order to provide adequate supply of fresh test solution. In addition, the following information is provided in published article: no degradation of Bisphenol A was detected in natural water system within 1 day. Therefore, RAC is of the opinion that the test concentrations could be maintained throughout the test.

RAC acknowledges that the study has some drawbacks which make it less reliable:

PFOS was identified in the test water. However, the idea of this study is to confirm the hypothesis that PFOS will increase the effects of Bisphenol A. As authors concluded this hypothesis was not confirmed even at presence of high PFOS concentrations. Therefore, RAC does not expect that low PFOS concentrations in test water would influence the results obtained for Bisphenol A.

Endpoint growth was used by DS for chronic classification. RAC is of the opinion that there are some uncertainties concerning this endpoint due to miscounting of numbers of fish per aquaria at the first time of sampling (30dpf), resulting in a slightly higher fish density throughout a two month period in one replicate (61 vs 44).

RAC considers this study as additional information.

Long-term toxicity studies with *X. laevis* (Levy et al., 2004, Pickford et al., 2010 and Pickford et al., 2003)

RAC recognizes that the study by Levy et al. (2004) has some shortcomings in relation to endpoint sex ratio which is considered for chronic classification by the DS. The following deficiencies were pointed out by the industry association (refer to RCOM) and in the Risk Assessment Report (2010): insufficient evidence of a concentration-response relationship, insufficient replication in the test design, statistical methodology used for analysis of the sex ratio data introduces statistical bias and absence of significant incidences of gonadal abnormalities at the histological level is not consistent with the presumed effect of bisphenol A on gonadal development (estrogenic activity of bisphenol A). Based on these deficiencies, the view of the RAC is that value 120 d NOEC of 0.0073 mg/L is not reliable to be considered for chronic classification purposes.

Species Sensitivity Distribution (SSD) approach

After the RAC consultation, RAC conducted the calculations of SSD (HC₅ value) for acute and chronic toxicity with online program MOSAIC_{SSD} (<https://mosaic.univ-lyon1.fr/ssd>).

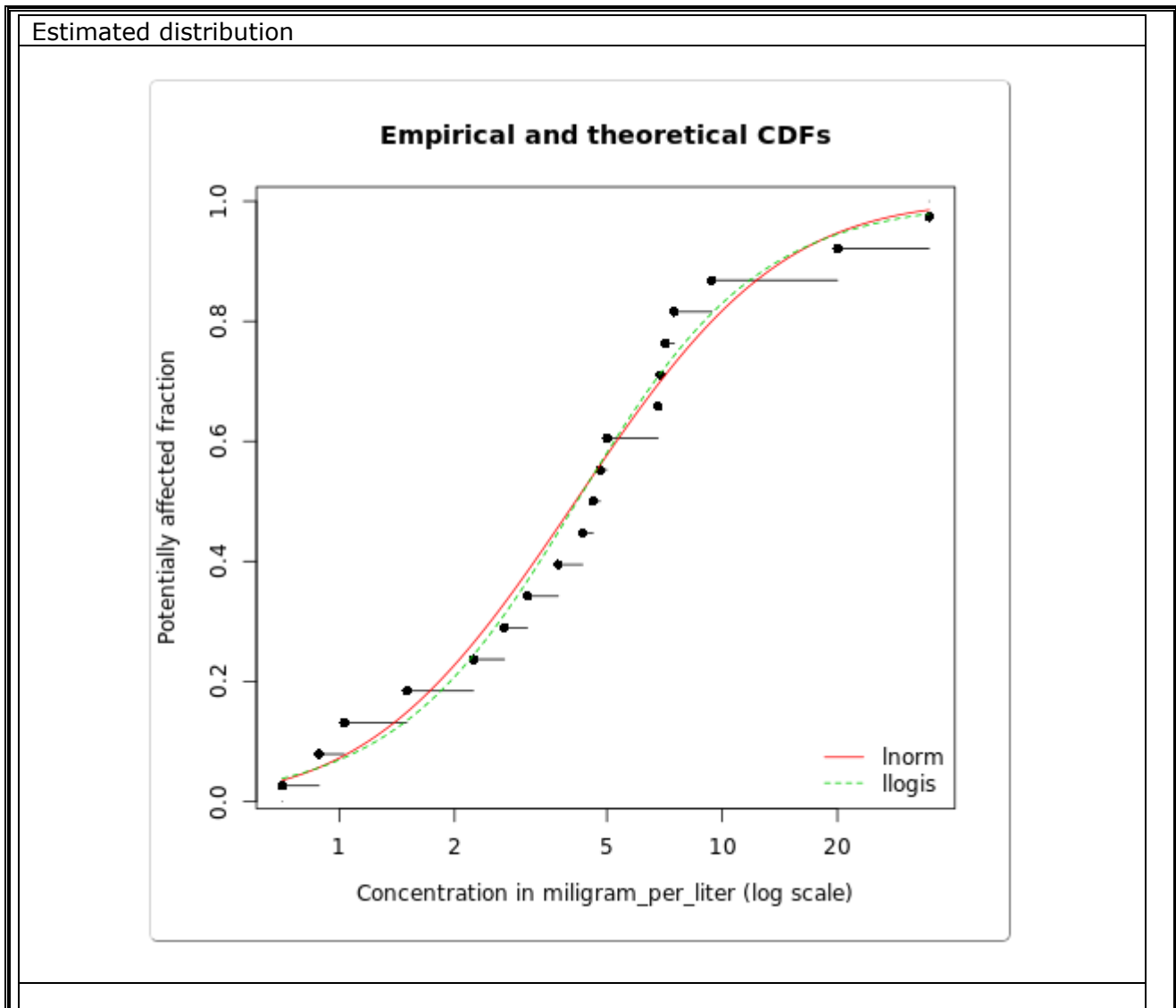
For the SSD calculation for acute toxicity, RAC has used the same acute toxicity values as the DS (presented in the table 3). An HC₅ of 0.83 mg/L was obtained with lower and upper limits of 0.44 and 1.7 mg/L, respectively.

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Table 3: Acute toxicity dataset used for calculation of SSD (HC₅ value)

Species and reference	Toxicity value (mg/L)
<i>Danio rerio</i> (McCormick <i>et al.</i> , 2011)	5
<i>Oryzias latipes</i> (Kashiwada <i>et al.</i> , 2002)	6.8
<i>Pimephales promelas</i> (Alexander <i>et al.</i> , 1988)	4.6
<i>Cyprinodon variegatus</i> (Dow Company, 1978; Emmittee 1978)	7.5
<i>Menidia menidia</i> (Alexander <i>et al.</i> 1988)	9.4
<i>Gammarus pulex</i> (Watts <i>et al.</i> , 2001)	1.5
<i>Americamysis bahia</i> (Hirano <i>et al.</i> , 2004)	1.03
<i>Artemia franciscana</i> (Castritsi-Catharios <i>et al.</i> , 2013)	34.7
<i>Tigriopus japonicus</i> (Marcial <i>et al.</i> , 2003)	4.32
<i>Acartia clausi</i> (Tato <i>et al.</i> , 2018)	0.885
<i>Chironomus tentans</i> (Springborn Smithers Lab., 2005)	2.7
<i>Marisa cornuarietis</i> (Mihaich <i>et al.</i> , 2009)	2.24
<i>Paracentrotus lividus</i> (Ozlem and Hatice, 2008)	0.71
<i>Hydra vulgaris</i> (Pascoe <i>et al.</i> , 2002)	6.9
<i>Pseudokirchneriella subcapitata</i> # (Alexander <i>et al.</i> , 1988)	3.10*
<i>Navicula incerta</i> (Liu <i>et al.</i> , 2010)	3.73
<i>Lemna gibba</i> (Putt 2003; Mihaich <i>et al.</i> , 2009)	20
<i>Rhinella arenarum</i> (Hutler <i>et al.</i> , 2014; Wolkowicz <i>et al.</i> , 2011)	7.1
<i>Xenopus laevis</i> (Iwamuro <i>et al.</i> , 2003)	4.8
SSD (HC₅) by Rapp with MOSAIC	0.83
* - There is an error in the CLH report. The EC ₅₀ value of 3.10 mg/L (Alexander <i>et al.</i> , 1988) is based upon biomass change and not reduction in growth rate; # - also known as <i>Selenastrum capricornutum</i> (with this name cited in CLH report)	

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The dataset used for the chronic toxicity SSD calculation by RAC is presented in table 4. Data used by RAC in SSD calculations are different from the one used by DS due to the following: mistakes were found in cited results, endpoint not relevant for chronic toxicity and some studies or results were found not reliable by RAC (see Supplemental Information part, **BD**). A HC₅ of 0.0012 mg/L was obtained with lower and upper limits of 0.00024 and 0.0072 mg/L, respectively.

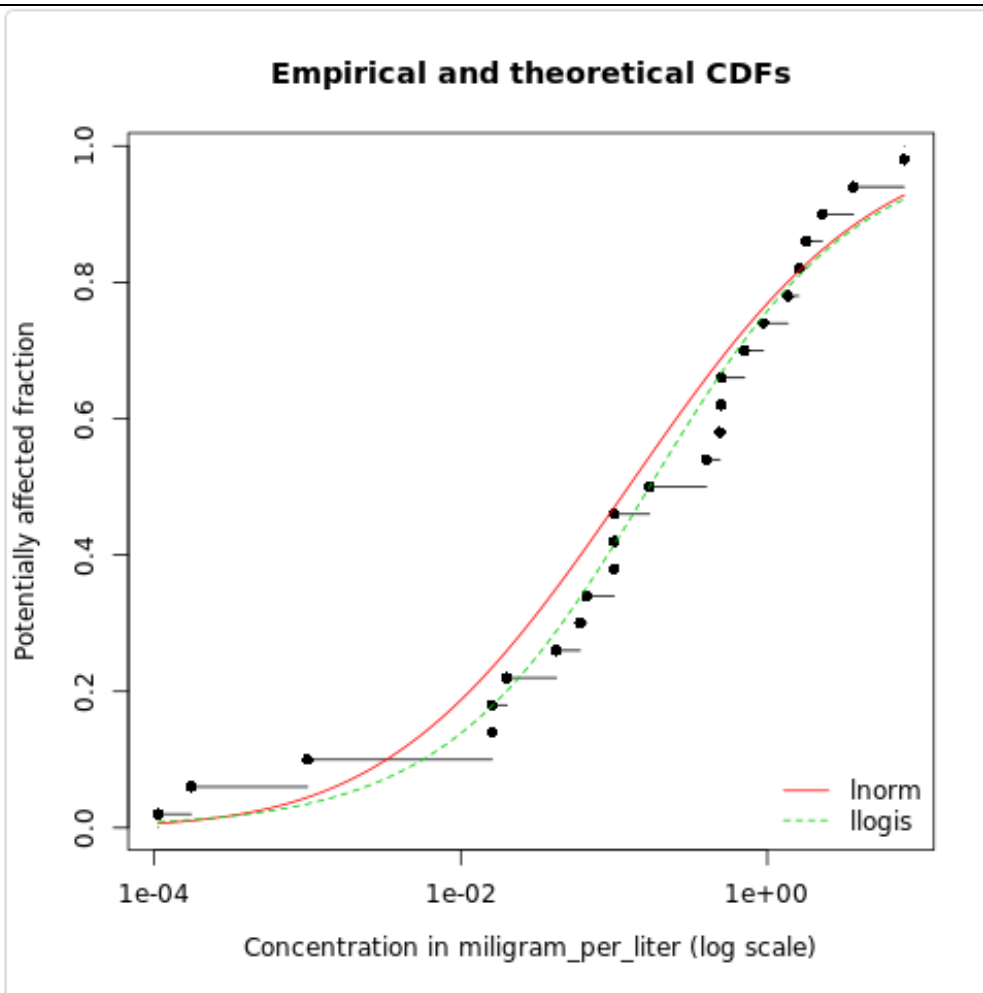
Table 4: Chronic toxicity dataset used for calculation of SSD (HC₅ value)

Species and reference	Toxicity value (mg/L)
<i>Cypinus carpio</i> (Bowmer & Gimeno, 2001)	0.016
<i>Danio rerio</i> (Chen <i>et al.</i> , 2017)	0.000174
<i>Oryzias latipes</i> (Sun <i>et al.</i> , 2014)	0.060
<i>Oncorhynchus mykiss</i> (Bayer AG 1999b)	3.64
<i>Pimephales promelas</i> (Sumpter <i>et al.</i> , 2001)	0.016
<i>Poecilia reticulata</i> (Kinnberg and Toft, 2003)	0.5
<i>Cyprinodon variegatus</i> (Mihaich 2018)	0.066
<i>Daphnia magna</i> (geomin)	2.28
<i>Ceriodaphnia dubia</i> (Tatarazako <i>et al.</i> , 2002)	0.94

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<i>Acartia tonsae</i> (Andersen <i>et al.</i> , 2001)	0.1
<i>Tigriopus japonicus</i> (Marcial <i>et al.</i> , 2003)	0.001
<i>Hyalella azteca</i> (Mihaich <i>et al.</i> , 2009)	0.49
<i>Americamysis bahia</i> (Mihaich <i>et al.</i> , 2018)	0.17
<i>Chironomus riparius</i> (Watts <i>et al.</i> , 2003)	0.1
<i>Marisa cornuarietis</i> (Oehlmann <i>et al.</i> 2006)	0.000106
<i>Potamopyrgus antipodarum</i> (Sieratowicz <i>et al.</i> , 2011)	0.02
<i>Physa acuta</i> (Sanches-Arguello <i>et al.</i> , 2012)	0.1
<i>Hemicentrotus pulcherrimus</i> (Kiyomoto <i>et al.</i> , 2005)	0.71
Heteromyenia sp (Hill <i>et al.</i> , 2002)	1.6
<i>Hydra vulgaris</i> (Pascoe <i>et al.</i> , 2002)	0.042
<i>Brachionus calyciflorus</i> (Mihaich <i>et al.</i> , 2009)	1.8
<i>Pseudokirchneriella subcapitata</i> (Alexander <i>et al.</i> 1988)	1.36
<i>Skeletonema costatum</i> (Alexander <i>et al.</i> 1988)	0.4
<i>Lemna gibba</i> (Putt, 2003; Mihaich <i>et al.</i> , 2009)	7.8
<i>Xenopus laevis</i> (Pickford, 2010)	0.5
SSD (HC₅) by RAC with MOSAIC_{SSD}	0.0012

Estimated distribution



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12 REFERENCES

- Alexander HC, Bartlett EA, Boggs GU (1985a): Bisphenol-A: Flow-through acute toxicity to the fathead minnow. Dow Company Report: ES-764.
- Alexander HC, Dill DC, Milazzo DP, Boggs GU (1985b). Bisphenol-A: Algal Toxicity Test. Dow Company Report: ES-762. 1985.
- Alexander HC, Milazzo DP and Boggs GU (1985c). Bisphenol-A: daphnid static acute toxicity test. Dow Company Report. ES-763.
- Alexander H.C., Dill D.C., Smith L.W., Guiney P.D., and Dorn P. (1988): Bisphenol A: Acute aquatic toxicity. *Environmental Toxicology and Chemistry* 7, 19-26
- Andersen H.R., Halling-Sorensen B., and Kusk K.O. (1999): A parameter for detecting estrogenic exposure in the copepod *Acartia tonsa*. *Ecotoxicol Environ Saf* 44 (1), 56-61. DOI: 10.1006/eesa.1999.1800
- Andersen H.R., Wollenberger L., Halling-Sorensen B., and Kusk K.O. (2001): Development of copepod nauplii to copepodites--a parameter for chronic toxicity including endocrine disruption. *Environ Toxicol Chem* 20 (12), 2821-2829. <http://www.ncbi.nlm.nih.gov/pubmed/11764166>
<http://onlinelibrary.wiley.com/store/10.1002/etc.5620201222/asset/5620201222ftp.pdf?v=1&t=iufj2vj4&s=0b40b082a4282cddcef7a8c2e2ee576fab4b5523>
- Baba K., Okada K., Kinoshita T., and Imaoka S. (2009): Bisphenol A disrupts Notch signaling by inhibiting gamma-secretase activity and causes eye dysplasia of *Xenopus laevis*. *Toxicol Sci* 108 (2), 344-355. DOI: 10.1093/toxsci/kfp025
- Benstead R.S. (2010): The Effects of the Natural Vertebrate Steroid 17 β -Oestradiol and the Xeno-Biotic Vertebrate Oestrogen Receptor Agonist Bisphenol-A on Reproduction in Selected Temperate Freshwater Gastropods: The Potential for (Neuro-) Endocrine Disruption., Brunel University. bura.brunel.ac.uk/bitstream/2438/4664/1/FulltextThesis.pdf
- Bhandari R.K., vom Saal F.S., and Tillitt D.E. (2015): Transgenerational effects from early developmental exposures to bisphenol A or 17 α -ethinylestradiol in medaka, *Oryzias latipes*. *Sci. Rep.* 5. DOI: 10.1038/srep09303
<http://www.nature.com/srep/2015/150320/srep09303/abs/srep09303.html#supplementary-information>
- Brennan S.J., Brougham C.A., Roche J.J., and Fogarty A.M. (2006): Multi-generational effects of four selected environmental oestrogens on *Daphnia magna*. *Chemosphere* 64 (1), 49-55. DOI: 10.1016/j.chemosphere.2005.11.046
- Caspers N. (1998): No estrogenic effects of Bisphenol A in *Daphnia magna* STRAUS. *Bull Environ Contam Toxicol* 61 (2), 143-148. <http://www.ncbi.nlm.nih.gov/pubmed/9702368>
- Castritsi-Catharios J., Syriou V., Miliou H., and Zouganelis G.D. (2013): Toxicity effects of bisphenol A to the nauplii of the brine shrimp *Artemia franciscana*. *Journal of Biological Research-Thessaloniki* 19, 38-45
- Chan W.K. and Chan K.M. (2012): Disruption of the hypothalamic-pituitary-thyroid axis in zebrafish embryo-larvae following waterborne exposure to BDE-47, TBBPA and BPA. *Aquat Toxicol* 108, 106-111. DOI: 10.1016/j.aquatox.2011.10.013
- Chen J., Saili K.S., Liu Y., Li L., Zhao Y., Jia Y., Bai C., Tanguay R.L., Dong Q., and Huang C. (2017): Developmental bisphenol A exposure impairs sperm function and reproduction in zebrafish. *Chemosphere* 169, 262-270. DOI: 10.1016/j.chemosphere.2016.11.089

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ISOPROPYLDENEDIPHENOL; BISPHENOL A

Chen J., Xiao Y., Gai Z., Li R., Zhu Z., Bai C., Tanguay R.L., Xu X., Huang C., and Dong Q. (2015): Reproductive toxicity of low level bisphenol A exposures in a two-generation zebrafish assay: Evidence of male-specific effects. *Aquat Toxicol* 169, 204-214. DOI: 10.1016/j.aquatox.2015.10.020

Chen M.-Y., Ike M., and Fujita M. (2002): Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. *Environmental Toxicology* 17 (1), 80-86.

<http://www3.interscience.wiley.com/journal/90011274/abstract>

<http://onlinelibrary.wiley.com/store/10.1002/tox.10035/asset/10035 ftp.pdf?v=1&t=hok78rja&s=f7eb03264ed7c6c72fb1abde63ecd5f7487eca90>

Duan Z., Zhu L., Zhu L., Kun Y., and Zhu X. (2008): Individual and joint toxic effects of pentachlorophenol and bisphenol A on the development of zebrafish (*Danio rerio*) embryo. *Ecotoxicol Environ Saf* 71 (3), 774-780. DOI: <https://doi.org/10.1016/j.ecoenv.2008.01.021>

ECHA (2017a): Guidance on the application of the CLP criteria

ECHA (2017b): Member State Committee Support Document for Identification of 4,4'-

Isopropylidenediphenol (Bisphenol A, BPA) as a Substance of Very High Concern because of its endocrine disrupting properties (Article 57(f)) causing probable serious effects to the environment which give rise to an equivalent level of concern to those of CMR and PBT/vPvB properties, date: 14.12.2017. European Chemicals Agency, <https://www.echa.europa.eu/web/guest/candidate-list-table/-/dislist/details/0b0236e180e22414>

European Commission (2003): European Union Risk Assessment Report 4,4'-ISOPROPYLDENEDIPHENOL (BISPHENOL-A). Office for Official Publications of the European Communities, Luxembourg

European Commission (2010): European Union Risk Assessment Report 4,4-ISOPROPYLENEDIPHENOL (Bisphenol-A) complete risk assessment in one document. Office for Official Publications of the European Communities. European Commission, Luxembourg

Forbes V.E., Selck H., Palmqvist A., Aufderheide J., Warbritton R., Pounds N., Thompson R., van der Hoeven N., and Caspers N. (2007): Does bisphenol A induce superfeminization in *Marisa cornuarietis*? Part I: intra- and inter-laboratory variability in test endpoints. *Ecotoxicol Environ Saf* 66 (3), 309-318. DOI: 10.1016/j.ecoenv.2006.10.014

Forbes V.E., Warbritton R., Aufderheide J., Van Der Hoeven N., and Caspers N. (2008): Effects of bisphenol a on fecundity, egg hatchability, and juvenile growth of *Marisa cornuarietis*. *Environ Toxicol Chem* 27 (11), 2332-2340. DOI: 10.1897/07-622.1

Fukuhori N., Kitano M., and Kimura H. (2005): Toxic effects of bisphenol A on sexual and asexual reproduction in *Hydra oligactis*. *Arch Environ Contam Toxicol* 48 (4), 495-500. DOI: 10.1007/s00244-004-0032-1

Heimeier R.A., Das B., Buchholz D.R., and Shi Y.B. (2009): The xenoestrogen bisphenol A inhibits postembryonic vertebrate development by antagonizing gene regulation by thyroid hormone. *Endocrinology* 150 (6), 2964-2973. DOI: 10.1210/en.2008-1503

Hill M., Stabile C., Steffen L.K., and Hill A. (2002): Toxic effects of endocrine disrupters on freshwater sponges: common developmental abnormalities. *Environ Pollut* 117 (2), 295-300.

<http://www.ncbi.nlm.nih.gov/pubmed/11916044>

Hirano M., Ishibashi H., Matsumura N., Nagao Y., Watanabe N., Watanabe A., Onikura N., Kishi K., and Arizono K. (2004): Acute toxicity responses of two crustaceans, *Americamysis bahia* and *Daphnia magna*, to endocrine disrupters. *Journal of Health Science* 50 (1), 97-100. DOI: 10.1248/jhs.50.97

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-
ISOPROPYLIDENEDIPHENOL; BISPHENOL A

- Honkanen J.O., Holopainen I.J., and Kukkonen J.V. (2004): Bisphenol A induces yolk-sac oedema and other adverse effects in landlocked salmon (*Salmo salar* m. sebago) yolk-sac fry. *Chemosphere* 55 (2), 187-196. http://ac.els-cdn.com/S0045653503010427/1-s2.0-S0045653503010427-main.pdf?_tid=3a6487c0-584a-11e3-9109-00000aab0f6b&acdnat=1385656338_8f3a9623cebea624d94d3bc7b75fa6a2
- Hutler W.I.R., Jorge H., and S. P.C.C. (2014): Stage-dependent toxicity of bisphenol a on *Rhinella arenarum* (anura, bufonidae) embryos and larvae. *Environ Toxicol* 29 (2), 146-154. DOI: doi:10.1002/tox.20781
- Ike M., Jin C.S., and Fujita M. (2000): Biodegradation of bisphenol A in the aquatic environment. *Water Science and Technology* 42, 31-38
- Iwamuro S., Sakakibara M., Terao M., Ozawa A., Kurobe C., Shigeura T., Kato M., and Kikuyama S. (2003): Teratogenic and anti-metamorphic effects of bisphenol A on embryonic and larval *Xenopus laevis*. *General and Comparative Endocrinology* 133 (2), 189-198. DOI: 10.1016/s0016-6480(03)00188-6
- Jemec A., Tisler T., Erjavec B., and Pintar A. (2012): Antioxidant responses and whole-organism changes in *Daphnia magna* acutely and chronically exposed to endocrine disruptor bisphenol A. *Ecotoxicol Environ Saf* 86, 213-218. DOI: 10.1016/j.ecoenv.2012.09.016
- Jeong S.W., Lee S.M., Yum S.S., Iguchi T., and Seo Y.R. (2013): Genomic expression responses toward bisphenol-A toxicity in *Daphnia magna* in terms of reproductive activity. *Molecular & Cellular Toxicology* 9 (2), 149-158
- Jobling S., Casey D., Rogers-Gray T., Oehlmann J., Schulte-Oehlmann U., Pawlowski S., Baunbeck T., Turner A.P., and Tyler C.R. (2004): Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquat Toxicol* 66 (2), 207-222. <http://www.ncbi.nlm.nih.gov/pubmed/15046010>
http://ac.els-cdn.com/S0166445X04000037/1-s2.0-S0166445X04000037-main.pdf?_tid=45d612d6-a0d1-11e6-9e56-00000aacb35d&acdnat=1478073316_c162d259b6f3879a74b66a6945756198
- Johnson I., Weeks J.M., and Kille P. (2005): Endocrine Disruption in Aquatic and Terrestrial Invertebrates - Final Report for the Department of the Environment, Food and Rural Affairs
- Kang I.J., Yokota H., Oshima Y., Tsuruda Y., Oe T., Imada N., Tadokoro H., and Honjo T. (2002): Effects of bisphenol a on the reproduction of Japanese medaka (*Oryzias latipes*). *Environ Toxicol Chem* 21 (11), 2394-2400. http://onlinelibrary.wiley.com/store/10.1002/etc.5620211119/asset/5620211119_ftp.pdf?v=1&t=hol5h50k&s=3594252449068f82cf72a7463f2104abad69f140
<http://www.ncbi.nlm.nih.gov/pubmed/12389919>
- Kang J.H. and Kondo F. (2002): Bisphenol A degradation by bacteria isolated from river water. *Archives of Environmental Contamination and Toxicology* 43 (3), 265-269. <http://www.scopus.com/scopus/inward/record.url?eid=2-s2.0-0036777653&partnerID=40>
http://download.springer.com/static/pdf/769/art%253A10.1007%252Fs00244-002-1209-0.pdf?auth66=1385885342_bdc04fb1686ca74538caa2faef8056f6&ext=.pdf
- Kang J.H. and Kondo F. (2005): Bisphenol A degradation in seawater is different from that in river water. *Chemosphere* 60 (9), 1288-1292. DOI: 10.1016/j.chemosphere.2005.01.058
- Karagiri K. (2004): Biodegradation Study of Bisphenol A by Microorganisms. Report no. 14294. Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, Unpublished Report
- Kashiwada S., Ishikawa H., Miyamoto N., Ohnishi Y., and Magara Y. (2002): Fish test for endocrine-disruption and estimation of water quality of Japanese rivers. *Water Res* 36 (8), 2161-2166. <http://ac.els->

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-
ISOPROPYLIDENEDIPHENOL; BISPHENOL A

cdn.com/S0043135401004067/1-s2.0-S0043135401004067-main.pdf?_tid=8a2b20ec-58cd-11e3-aa10-0000aacb361&acdnat=1385712736_c7b2ba50d7cda76bad9910bb65b603ad

Keiter S., Baumann L., Farber H., Holbech H., Skutlarek D., Engwall M., and Braunbeck T. (2012): Long-term effects of a binary mixture of perfluorooctane sulfonate (PFOS) and bisphenol A (BPA) in zebrafish (*Danio rerio*). *Aquat Toxicol* 118-119, 116-129. DOI: 10.1016/j.aquatox.2012.04.003

Kinnberg K. and Toft G. (2003): Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). *Ecotoxicol. Environ Saf.* 54 (1), 16-24.
<http://www.ncbi.nlm.nih.gov/pubmed/12547630>

Kiyomoto M., Kikuchi A., Unuma T., and Yokota Y. (2005): Effects of ethynylestradiol and bisphenol A on the development of sea urchin embryos and juveniles. *Marine Biology* 149 (1), 57-63. DOI: 10.1007/s00227-005-0208-x

Klečka G.M., Gonsior S.J., West R.J., Goodwin P.A., and Markham D.A. (2001): Biodegradation of bisphenol A in aquatic environments: river die-away. *Environ Toxicol Chem* 20 (12), 2725-2735.
<http://www.ncbi.nlm.nih.gov/pubmed/11764155>

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/rtp.1996.1076

Lahnsteiner F., Berger B., Kletzl M., and Weismann T. (2005): Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, *Salmo trutta f. fario*. *Aquat Toxicol* 75 (3), 213-224. DOI: 10.1016/j.aquatox.2005.08.004

Levy G.L., I.; Krüger, A.; Kloas, W. (2004): Bisphenol A induces feminization in *Xenopus laevis* tadpoles. *Environmental Research* 94 (1), 102-111. DOI: 10.1016/s0013-9351(03)00086-0

Li M.-H. (2013): Acute toxicity of industrial endocrine-disrupting chemicals, natural and synthetic sex hormones to the freshwater planarian, *Dugesia japonica*. *Toxicological & Environmental Chemistry* 95 (6), 984-991. DOI: 10.1080/02772248.2013.840376

Li R., Liu Y., Chen G., F. Y. Tam N., Shin P., Cheung S.G., and Luan T. (2008): Physiological responses of the alga *Cyclotella caspia* to Bisphenol A exposure. DOI: 10.1515/BOT.2008.050

Liu Y., Guan Y., Gao Q., Tam N.F.Y., and Zhu W. (2010): Cellular responses, biodegradation and bioaccumulation of endocrine disrupting chemicals in marine diatom *Navicula incerta*. *Chemosphere* 80 (5), 592-599. DOI: <https://doi.org/10.1016/j.chemosphere.2010.03.042>

Liu Y., Tam N.F., Guan Y., Yasojima M., Zhou J., and Gao B. (2011): Acute toxicity of nonylphenols and bisphenol A to the embryonic development of the abalone *Haliotis diversicolor supertexta*. *Ecotoxicology* 20 (6), 1233-1245. DOI: 10.1007/s10646-011-0672-7

Mansilha C., Silva P., Rocha S., Gameiro P., Domingues V., Pinho C., and Ferreira I.M. (2013): Bisphenol A migration from plastic materials: direct insight of ecotoxicity in *Daphnia magna*. *Environ Sci Pollut Res Int* 20 (9), 6007-6018. DOI: 10.1007/s11356-013-1614-0

Marcial H.S., Hagiwara A., and Snell T.W. (2003): Estrogenic compounds affect development of harpacticoid copepod *Tigriopus japonicus*. *Environmental Toxicology and Chemistry* 22 (12), 3025-3030.
<http://www3.interscience.wiley.com/cgi-bin/fulltext/122674072/PDFSTART>

http://onlinelibrary.wiley.com/store/10.1897/02-622/asset/5620221225_ft.pdf?v=1&t=hol5zwy9&s=25eec40da620772cdf4f289760c36220b80b628
<http://www.ncbi.nlm.nih.gov/pubmed/14713045>

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-
ISOPROPYLIDENEDIPHENOL; BISPHENOL A

- McCormick J.M., Van Es T., Cooper K.R., White L.A., and Haggblom M.M. (2011): Microbially mediated O-methylation of bisphenol A results in metabolites with increased toxicity to the developing zebrafish (*Danio rerio*) embryo. *Environ Sci Technol* 45 (15), 6567-6574. DOI: 10.1021/es200588w
- Mihaich E., Rhodes J., Wolf J., van der Hoeven N., Dietrich D., Hall A.T., Caspers N., Ortego L., Staples C., Dimond S., and Hentges S. (2012): Adult fathead minnow, *Pimephales promelas*, partial life-cycle reproductive and gonadal histopathology study with bisphenol A. *Environmental Toxicology and Chemistry* 31 (11), 2525-2535. DOI: 10.1002/etc.1976
- Mihaich E., Staples C., Ortego L., Klecka G., Woelz J., Dimond S., and Hentges S. (2018): Life-cycle studies with 2 marine species and bisphenol A: The mysid shrimp (*Americamysis bahia*) and sheepshead minnow (*Cyprinodon variegatus*). *Environ Toxicol Chem* 37 (2), 398-410. DOI: 10.1002/etc.3957
- Mihaich E.M., Friederich U., Caspers N., Hall A.T., Klecka G.M., Dimond S.S., Staples C.A., Ortego L.S., and Hentges S.G. (2009): Acute and chronic toxicity testing of bisphenol A with aquatic invertebrates and plants. *Ecotoxicology and Environmental Safety*. <http://www.scopus.com/scopus/inward/record.url?eid=2-s2.0-62749120308&partnerID=40>
- MITI (1992): Biodegradation and Bioaccumulation of Existing Chemical Substances under the Chemical Substance Control Law. National Institute of Technology and Evaluation, Japan.
- Mu X., Rider C.V., Hwang G.S., Hoy H., and LeBlanc G.A. (2005): COVERT SIGNAL DISRUPTION: ANTI-ECDYSTEROIDAL ACTIVITY OF BISPHENOL A INVOLVES CROSS TALK BETWEEN SIGNALING PATHWAYS. *Environ Tox Chem* 24 (1), 146-152
- Oehlmann J., Schulte-Oehlmann U., Bachmann J., Oetken M., Lutz I., Kloas W., and Ternes T.A. (2006): Bisphenol A induces superfeminization in the ramshorn snail *Marisa cornuarietis* (Gastropoda: Prosobranchia) at environmentally relevant concentrations. *Environ Health Perspect* 114 Suppl 1, 127-133. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1874184/pdf/ehp0114s1-000127.pdf>
- Oehlmann J., Schulte-Oehlmann U., Tillmann M., and Markert B. (2000): Effects of Endocrine Disruptors on Prosobranch Snails (Mollusca: Gastropoda) in the Laboratory. Part I: Bisphenol A and Octylphenol as Xeno-Estrogens. *Ecotoxicology* 9 (6), 383-397. <http://www.springerlink.com/content/j06w54218215246m/fulltext.pdf>
http://download.springer.com/static/pdf/896/art%253A10.1023%252FA%253A1008972518019.pdf?auth66=1385886859_d96860f8a7b73cf2571947f0f070d2ac&ext=.pdf
- Ozlem C.A. and Hatice P. (2008): Effects of bisphenol A on the embryonic development of sea urchin (*Paracentrotus lividus*). *Environ Toxicol* 23 (3), 387-392. DOI: 10.1002/tox.20349
- Pascoe D., Carroll K., Karntanut W., and Watts M.M. (2002): Toxicity of 17alpha-ethinylestradiol and bisphenol A to the freshwater Cnidarian *Hydra vulgaris*. *Arch Environ Contam Toxicol* 43 (1), 56-63. DOI: 10.1007/s00244-001-0016-3
- Pickford D.B. (2010): Screening chemicals for thyroid-disrupting activity: A critical comparison of mammalian and amphibian models. *Crit Rev Toxicol* 40 (10), 845-892. DOI: 10.3109/10408444.2010.494250
- Pickford D.B., Hetheridge M.J., Caunter J.E., Tilghman Hall A., and Hutchinson T.H. (2003): Assessing chronic toxicity of bisphenol A to larvae of the African clawed frog (*Xenopus laevis*) in a flow-through exposure system. *Chemosphere* 53 (3), 223-235. DOI: 10.1016/s0045-6535(03)00308-4
- Ratte T.H. (2015): Statistical Analysis of a Laboratory Study about the Effects of Bisphenol A on the Reproduction of the Ramshorn snail *Marisa cornuarietis* (Mesogastropoda: Ampullariidae). UBA-Texte

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-ISOPROPYLIDENEDIPHENOL; BISPHENOL A

27/2015. RWTH Aachen University. Umweltbundesamt, Aachen.

<http://www.umweltbundesamt.de/publikationen/statistical-analysis-of-a-laboratory-study-about>

Sanchez-Arguello P., Aparicio N., and Fernandez C. (2012): Linking embryo toxicity with genotoxic responses in the freshwater snail *Physa acuta*: single exposure to benzo(a)pyrene, fluoxetine, bisphenol A, vinclozolin and exposure to binary mixtures with benzo(a)pyrene. *Ecotoxicol Environ Saf* 80, 152-160. DOI: 10.1016/j.ecoenv.2012.02.029

Segner H., Navas J.M., Schäfers C., and Wenzel A. (2003): Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo. *Ecotoxicology and Environmental Safety* 54 (3), 315-322. DOI: doi: DOI: 10.1016/S0147-6513(02)00040-4

Shioda T. and Wakabayashi M. (2000): Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). *Chemosphere* 40 (3), 239-243. <http://www.ncbi.nlm.nih.gov/pubmed/10665413>

Sieratowicz A., Stange D., Schulte-Oehlmann U., and Oehlmann J. (2011): Reproductive toxicity of bisphenol A and cadmium in *Potamopyrgus antipodarum* and modulation of bisphenol A effects by different test temperature. *Environ Pollut* 159 (10), 2766-2774. DOI: 10.1016/j.envpol.2011.05.012

Sohoni P., Tyler C.R., Hurd K., Caunter J., Hetheridge M., Williams T., Woods C., Evans M., Toy R., Gargas M., and Sumpter J.P. (2001): Reproductive effects of long-term exposure to Bisphenol A in the fathead minnow (*Pimephales promelas*). *Environ Sci Technol* 35 (14), 2917-2925. <http://pubs.acs.org/doi/pdfplus/10.1021/es000198n>

Stasinakis A.S., Petalas A.V., Mamais D., and Thomaidis N.S. (2008): Application of the OECD 301F respirometric test for the biodegradability assessment of various potential endocrine disrupting chemicals. *Bioresour Technol* 99 (9), 3458-3467. DOI: 10.1016/j.biortech.2007.08.002

Stone C.M. and Watkinson R.J. (1983): Diphenylol Propane: An Assessment of Ready Biodegradability. Research Report SBGR.83.425. Shell Group

Sun L., Lin X., Jin R., Peng T., Peng Z., and Fu Z. (2014): Toxic effects of bisphenol A on early life stages of Japanese medaka (*Oryzias latipes*). *Bull Environ Contam Toxicol* 93 (2), 222-227. DOI: 10.1007/s00128-014-1298-2

Tatarazako N., Takao Y., Kishi K., Onikura N., Arizono K., and Iguchi T. (2002): Styrene dimers and trimers affect reproduction of daphnid (*Ceriodaphnia dubia*). *Chemosphere* 48 (6), 597-601. <http://www.ncbi.nlm.nih.gov/pubmed/12143934>

http://ac.els-cdn.com/S0045653502001194/1-s2.0-S0045653502001194-main.pdf?_tid=9cd31382-a0fd-11e6-b9b3-00000aacb362&acdnat=1478092360_08605375f17676125e4dcbf57322f5ac

Tato T., Salgueiro-Gonzalez N., Leon V.M., Gonzalez S., and Beiras R. (2018): Ecotoxicological evaluation of the risk posed by bisphenol A, triclosan, and 4-nonylphenol in coastal waters using early life stages of marine organisms (*Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Acartia clausi*). *Environ Pollut* 232, 173-182. DOI: 10.1016/j.envpol.2017.09.031

van der Hoeven N. (2005): Experiments on the effect of BPA on the snail species *Marisa cornuarietis* as described in three papers by Oehlmann: Evaluation of the applied statistics and analysis of the raw data, date: 14.10.2005. ECOSTAT

Watts M.M., Pascoe D., and Carroll K. (2001): Survival and precopulatory behaviour of *Gammarus pulex* (L.) exposed to two xenoestrogens. *Water Res* 35 (10), 2347-2352. <http://www.ncbi.nlm.nih.gov/pubmed/11394767>

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-
ISOPROPYLIDENEDIPHENOL; BISPHENOL A

- Watts M.M., Pascoe D., and Carroll K. (2003): Exposure to 17[alpha]-ethinylestradiol and bisphenol A — effects on larval moulting and mouthpart structure of *Chironomus riparius*. *Ecotoxicol Environ Saf* 54, 207-215
- West R.J., Goodwin P.A., and Klecka G.M. (2001): Assessment of the Ready Biodegradability of Bisphenol A. *Bulletin of Environmental Contamination and Toxicology* 67 (1), 0106-0112. DOI: 10.1007/s00128-001-0097-8
- Yokota H., Tsuruda Y., Maeda M., Oshima Y., Tadokoro H., Nakazono A., Honjo T., and Kobayashi K. (2000): Effect of bisphenol A on the early life stage in Japanese medaka (*Oryzias latipes*). *Environmental Toxicology and Chemistry* 19 (7), 1925-1930. CCC:000087674600030
<http://onlinelibrary.wiley.com/store/10.1002/etc.5620190730/asset/5620190730 ftp.pdf?v=1&t=hol80mne&s=5412ed3b555925e725d165df15a2595265fe998e>
- Zhou J., Zhu X.S., and Cai Z.H. (2011): The impacts of bisphenol A (BPA) on abalone (*Haliotis diversicolor supertexta*) embryonic development. *Chemosphere* 82 (3), 443-450. DOI: 10.1016/j.chemosphere.2010.09.056
- Bayer AG, (1999a). Fish, prolonged toxicity test (*Brachydanio rerio*) 14 day study of Bisphenol-A. Study Number 707 A/98 Fl.
- Bayer AG (1999b). Fish, juvenile growth test (*Oncorhynchus mykiss*) of Bisphenol-A. Study Number: 707 A/98FF.
- Bowmer T and Gimeno S, (2001). The effects of Bisphenol-A on the development of the male carp reproductive tract when exposed during sexual differentiation – implications for test design and risk assessment. Unpublished TNO Report.
- Dow Company (1978). The acute toxicity of Parabis A and Bisphenol-A to the sheepshead minnow, *Cyprinodon variegatus*. Dow Company Report, TWC 102.
- Japanese Ministry of the Environment (2006). Results of Assay and Tests in Evaluation of the Endocrine Disrupting Activities in Fish (Medaka), 26 January 2006.
http://www.env.go.jp/en/chemi/ed/rt_medaka.pdf
- Lee, MR (2010): Bisphenol A – Life-cycle toxicity test with mysids (*Americamysis bahia*) following draft OPPTS guideline 850.1350. – Report No. 13943.6104. Springborn Laboratories, Inc., Wareham, MA, USA.
- Oehlmann, J., Schulte-Oehlmann, U., Bachmann, J., Oetken, M., Lutz, I., Kloas, W., and Ternes, T.A.. (2006b). Effects of BPA in Snails: Oehlmann et al. *respond. Environmental Health Perspectives* 114 (6): A341-342
- Putt AE (2003): Bisphenol A – 7 day toxicity to duckweed, *Lemna gibba*, under static renewal conditions. Springborn Smithers Laboratories Study No. 13796.6101 submitted to the American Plastics Council.
- ABC Laboratories (2008): Bisphenol A: Partial Life-Cycle Toxicity Test with the fathead minnow, *Pimephales promelas*. Study Number 48972. ABC Laboratories, Inc., Columbia, MO, USA.
- Springborn Smithers Lab. (2009): Bisphenol A – Acute toxicity to sheepshead minnow (*Cyprinodon variegates*) under flow-through conditions, following OPPTS Draft Guideline 850.1075 and OECD Guideline 203. Springborn Smithers Laboratories Study Number 13943.6103.
- Springborn Smithers Lab. (2005): Bisphenol A (BPA) – Acute toxicity to midges (*Chironomus tentans*) under flow-through conditions. Springborn Smithers Laboratories Study No. 13796.6107 submitted to the American Plastics Council.
- Springborn Smithers (2006a): Bisphenol A (BPA) - Chronic Toxicity to Amphipods (*Hyalella azteca*) Under Flow-Through Conditions. Unpublished report, No. 13796.6106

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-
ISOPROPYLIDENEDIPHENOL; BISPHENOL A

- Springborn Smithers (2006b): Bisphenol A (BPA) - Chronic Toxicity to Rotifers (*Brachionus calyciflorus*) Under Static Conditions, Unpublished report, No. 13796.6108.
- Stephenson RR (1983): Diphenylol propane: Acute toxicity to *Daphnia magna* and *Selenastrum capricornutum*. Shell Group Research Report SBGR.83.216
- Sumpter JP, Tyler CR, Sherazi A (2001). Bisphenol-A: Multigeneration study with the fathead minnow (*Pimephales promelas*). Brunel University.
- Warbritton R., Forbes V. and van der Heoven N. (2007b): Bisphenol A: Effects on Fecundity of *Marisa cornuarietis* under flow-through conditions at twenty-two degrees Celsius. – ABC Study No. 61028. ABC Laboratories, Columbia, MO, USA.
- Warbritton R., Forbes V. and van der Heoven N. (2007a): Bisphenol A: Effects on Fecundity, Hatchability, and Growth of *Marisa cornuarietis* under flow-through conditions at twenty-five degrees Celsius (Phase III Definitive Toxicity Test). – ABC Study No. 49820. ABC Laboratories, Columbia, MO, USA.
- Springborn Smithers (2010): Bisphenol A (BPA) – Full Life-cycle toxicity test with sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions, following OPPTS Guideline 850.1500. – Report No. 13943.6105. Springborn Smithers Laboratories, Inc, Wareham, MA, USA.