Committee for Risk Assessment

RAC

Annex 1

Background document

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

hexyl 2-(1-(diethylaminohydroxyphenyl) methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate

EC Number: 443-860-6

CAS Number: 302776-68-7

CLH-O-0000001412-86-253/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

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate

EC Number: **443-860-6**

CAS Number: **302776-68-7**

Index Number: **607-693-00-4**

Contact details for dossier submitter:

Federal Institute for Occupational Saftety and Health (BAuA)
Friedrich-Henkel-Weg 1-25
44149 Dortmund
Germany
Chemg@baua.bund.de

Version number: 1.0 **Date: October 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYL 2-(1-(DIETHYLAMINOHYDROXYPHENYL) METHANOYL)BENZOATE; HEXYL 2-[4-(DIETHYLAMINO)-2-HYDROXYBENZOYL]BENZOATE

CONTENTS

1 IDENTITY OF THE SUBSTANCE 1

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 1
1.2 COMPOSITION OF THE SUBSTANCE 1

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 2

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 2

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 3

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 4

5 IDENTIFIED USES 4

6 DATA SOURCES 4

7 PHYSICOCHEMICAL PROPERTIES 4

11 EVALUATION OF ENVIRONMENTAL HAZARDS 6

11.1 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES 6
11.1.1 Ready biodegradability 6
11.1.2 Hydrolysis 6
11.1.3 Other convincing scientific evidence 6
11.1.3.1 Photochemical degradation 6
11.2 ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION 6
11.3 BIOACCUMULATION 6
11.3.1 Estimated bioaccumulation 7
11.3.2 Measured partition coefficient and bioaccumulation test data 7
11.4 ACUTE AQUATIC HAZARD 8
11.4.1 Acute (short-term) toxicity to fish 8
11.4.2 Acute (short-term) toxicity to aquatic invertebrates 8
11.4.3 Acute (short-term) toxicity to algae or other aquatic plants 8
11.5 LONG-TERM AQUATIC HAZARD 9
11.5.1 Chronic toxicity to fish 9
11.5.2 Chronic toxicity to aquatic invertebrates 9
11.5.3 Chronic toxicity to algae or other aquatic plants 10
11.6 COMPARISON WITH THE CLP CRITERIA 10
11.6.1 Acute aquatic hazard 10
11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation) 11
11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS 11

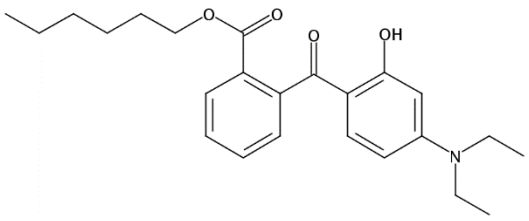
12 REFERENCES 26

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYL 2-(1-(DIETHYLAMINOHYDROXYPHENYL) METHANOYL)BENZOATE; HEXYL 2-[4-(DIETHYLAMINO)-2-HYDROXYBENZOYL]BENZOATE

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)	Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate
Other names (usual name, trade name, abbreviation)	Uvinul A Plus; Diethylamino hydroxybenzoyl hexyl benzoate; Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexyl ester
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	443-860-6
EC name (if available and appropriate)	hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate
CAS number (if available)	302776-68-7
Other identity code (if available)	INCI: Diethylamino hydroxybenzoyl hexyl benzoate
Molecular formula	$C_{24}H_{31}NO_4$
Structural formula	
SMILES notation (if available)	CCCCCCOC(=O)c1ccccc1C(=O)c2ccc(N(CC)CC)cc2O
Molecular weight or molecular weight range	397.51 g/mol

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 3.1 (CLP)	Current self-classification and labelling (CLP)
hexyl 2-(1-(diethylamino hydroxyphenyl)methanoyl) benzoate (CAS: 302776-68-7; EC: 443-860-6)	80-100	Aquatic Chronic 4; H413	Aquatic Chronic 4; H413 Not Classified

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	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	607-693-00-4	hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate	443-860-6	302776-68-7	Aquatic Chronic 4	H413	-	H413			
Dossier submitters proposal		hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate			**Modify:** Aquatic Chronic 4 to Aquatic Chronic 1	**Modify:** H413 to H410	**Add:** GHS09 Wng	**Modify:** H413 to H410		**Add:** M = 1000	
Resulting Annex VI entry if agreed by RAC and COM					Aquatic Chronic 1	H410	GHS09 Wng	H410		M = 1000	

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives		No
Flammable gases (including chemically unstable gases)		No
Oxidising gases		No
Gases under pressure		No
Flammable liquids		No
Flammable solids		No
Self-reactive substances		No
Pyrophoric liquids		No
Pyrophoric solids		No
Self-heating substances		No
Substances which in contact with water emit flammable gases		No
Oxidising liquids		No
Oxidising solids		No
Organic peroxides		No
Corrosive to metals		No
Acute toxicity via oral route		No
Acute toxicity via dermal route		No
Acute toxicity via inhalation route		No
Skin corrosion/irritation		No
Serious eye damage/eye irritation		No
Respiratory sensitisation		No
Skin sensitisation		No
Germ cell mutagenicity		No
Carcinogenicity		No
Reproductive toxicity		No
Specific target organ toxicity-single exposure		No
Specific target organ toxicity-repeated exposure		No
Aspiration hazard		No
Hazardous to the aquatic environment		**Yes**
Hazardous to the ozone layer		No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The harmonised classification and labelling of Uvinul A Plus (Aquatic Chronic 4) was included in Annex VI of the CLP-Regulation with the 1st ATP (Commission Regulation (EC) No 790/2009).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

Change in existing entry due to changes in the criteria (2.ATP)
Disagreement by DS with current self-classification

5 IDENTIFIED USES

This substance is used in the following products: cosmetics and personal care products.

6 DATA SOURCES

REACH registration dossier (04/2017)

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid	REACH registration dossier	-
Melting/freezing point	54 °C at 1013.0 hPa	REACH registration dossier	experimental result [OECD Guideline 102 (Melting point/Melting Range): thermal analysis]
Boiling point	substance decomposes at 314 °C before boiling	REACH registration dossier	experimental result [EU Method A.2 (Boiling Temperature): dynamic method]
Relative density	1.16 at 20 °C	REACH registration dossier	experimental result [OECD Guideline 109 (Density of Liquids and Solids): pycnometer method]
Vapour pressure	2.9 10^{-8} hPa at 20 °C	REACH registration dossier	experimental result [EU Method A.4 (Vapour Pressure): effusion method]
Surface tension	-	REACH registration dossier	n.a. (The water solubility is below 1 mg/L at 20°C.)
Water solubility	16 µg/l at 20 °C (pH = 6.9)	REACH registration dossier	experimental result [OECD Guideline 105 (Water Solubility): column elution method]
Partition coefficient n-octanol/water	log Pow = 6.2 at 24 ° C	REACH registration dossier	experimental result [EU Method A.8 (Partition Coefficient): HPLC method]
Flash point			
Flammability			
Explosive properties			
Self-ignition temperature			
Oxidising properties			

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYL 2-(1-(DIETHYLAMINOHYDROXYPHENYL) METHANOYL)BENZOATE; HEXYL 2-[4-(DIETHYLAMINO)-2-HYDROXYBENZOYL]BENZOATE

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Granulometry	D10= 230.6 µm; D50= 1247.8 µm; D90= 1646.0 µm	REACH registration dossier	experimental result [Laser diffraction method according to ISO 13320-1: volumetric distribution]
Stability in organic solvents and identity of relevant degradation products	-		n.a. (The stability of the substance is not considered as critical.)
Dissociation constant	-		n.a. (The substance is not soluble in water.)
Viscosity	-		n.a. (Substance is a solid.)

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 6: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD Guideline 301 F (Manometric Respirometry Test)	2-5 % (O_2 consumption) after 28 days reference substance: 80-90 % after 14 days	Rel. 2 GLP study	(BASF, 2001b)

11.1.1 Ready biodegradability

Ready biodegradation of Uvinul A Plus was investigated in a study according to OECD Guideline 301 F using 30 mg/L inoculum (domestic activated sludge, non-adapted) and 100 mg/L test substance (BASF, 2001b). After 28 days 2-5 % degradation was observed. The percentage degradation of the reference substance (aniline) has reached the pass level after 14 days (80-90%). The test was performed at a pH-value of 7.3-7.4. No further details on this study are available in the REACH registration dossier.

Uvinul A Plus is not readily biodegradable.

11.1.2 Hydrolysis

No experimental data available.

Half-lives of 250 days at pH 8 and 6.9 years at pH 7 were estimated by EPI Suite HYDROWIN (v2.00).

11.1.3 Other convincing scientific evidence

No data available.

11.1.3.1 Photochemical degradation

A rate constant of 0.0000000002252403 cm³/molecule*sec and a half-life in the atmosphere of 1.7 hours for Uvinul A Plus was predicted by a calculation assuming a 24 hour day and an OH-radical concentration of 5.0E+05 molecules/cm³ (SRC AOP v1.92, 2007) (ECHA, 2017). Hence, if the substance will be exposed to air, it will be rapidly degraded by photochemical degradation. Nevertheless, based on Henry´s law constant (see chapter 11.2) the substance will not evaporate from water surface to air.

11.2 Environmental fate and other relevant information

The adsorption of the substance was tested by OECD Guideline 121. Based on a log Koc of 5.1 (23°C) adsorption to sediment and soil is expected (BASF, 2010).

Henry´s law constant of 0.000019 Pa·m³/mol was calculated by SRC HENRYWIN (v3.10). The substance has a very low potential to evaporate from water surface to air (ECHA, 2017).

11.3 Bioaccumulation

Table 7: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD Guideline 305 *Danio rerio* Uptake period = 28 days Depuration period = 16-21	1.0 µg/L exposure concentration: BCF_{ss} = 215.4 BCF_k = 204.6	Rel. 2 GLP-study	(BASF, 2006)

Method	Results	Remarks	Reference
days	0.1 µg/L exposure concentration: BCF_{ss} = 126.8 BCF_k = 120.3 Lipid and growth corrected: BCF = 360 (1.0 µg/L exposure concentration) BCF = 230 (0.1 µg/L exposure concentration)		
OECD Guideline 305 *Danio rerio* Uptake period = 21 days Depuration period = 7 days	BCF_k = 225.6 BCF_{ss} = 193.4	Rel. 2	(BASF, 2005)

BCF_{ss} = bioconcentration factor based on steady state concentrations;
BCF_k = bioconcentration factor based on kinetic modelling

11.3.1 Estimated bioaccumulation

11.3.2 Measured partition coefficient and bioaccumulation test data

A log Kow value of 6.2 (24 °C) was determined by EU Method A.8 (HPLC method) (BASF, 2000c). The study was performed without adjustment of pH value. No further information on study design is available.

The bioconcentration factor of Uvinul A Plus was measured for *Danio rerio* using OECD Guideline 305. The study was carried out in a flow-through system and two exposure concentrations (0.1 and 1 µg/L, nominal) were assessed over an uptake phase of 28 days and a depuration phase of 16 days (1 µg/L) and 21 days (0.1 µg/L). ^{14}C-radiolabelled test substance was used. An aqueous stock solution of 100 µg/L was used. It was prepared by dissolving 9.30 mg test substance in 120 mL acetone. The concentration in fish reached steady state within 7 days in both concentration groups. Based on the steady state concentrations the bioconcentration factor BCF_{ss} in whole fish was 126.8 in the lower concentration and 215.4 in the higher concentration. Based on kinetic modelling the bioconcentration factor BCF_k in whole fish was 120.3 in the lower and 204.6 in the higher concentration. In conclusion the bioconcentration factor for Uvinul A Plus was 166.8 based on the mean of BCF_{ss} and BCF_k in both test concentrations. During the depuration phase the half-life time for the test substance in fish was 0.9 days in the low and 1.4 days in the high concentration. Approximately 90 % of the steady state-concentration of the test substance was excreted after 3.1 days in the low concentration and after 4.8 days in the high concentration. The lipid content was in the range between 3.01 and 4.62 % over the whole uptake and elimination period but no lipid corrected BCFs were provided. A growth corrected BCF was not calculated. However, statistical estimation by applying the R-package of the revised OECD 305 Guidance Doc (2016) yields a lipid and growth corrected BCF of 360 (mean lipid content of 3.84 %; 4 % growth rate per day) for the higher exposure concentration and 230 for the lower exposure concentration.

The result of the first study is supported by a screening study according to OECD Guideline 305. The study was carried out in a flow-through system and an exposure concentration of 1 µg/L (nominal) over an uptake period of 21 days followed by a depuration period of 7 days. ^{14}C-radiolabelled test substance was used. An aqueous stock solution of 100 µg/L was used. It was prepared by dissolving 8.75 mg test substance in 100 mL acetone. The BFC-values in whole fish are considered to be 225.6 based on kinetic data and 193.4 based on steady state concentration. The time to steady state was approximately 1 day. During the depuration phase the half-life of the test substance in fish was 1.17 days (DT90 = 3.9 days).

11.4 Acute aquatic hazard

Table 8: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 203	*Danio rerio*	CAS 302776-68-7	96h-LC_{50} > 100 mg/L (nominal)	Rel. 2 (registrant rel. 1) GLP-study	(BASF, 2000b)
OECD 202	*Daphnia magna*	CAS 302776-68-7	48h-EC_{50} > 100 mg/L (nominal)	Rel. 2 (registrant rel. 1) GLP-study	(BASF, 2000a)
OECD 201	*Desmodesmus subspicatus*	CAS 302776-68-7	72h-E_rC_{50} > 100 mg/L (nominal)	Rel. 2 (registrant rel. 1) GLP-study	(BASF, 2001a)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.4.1 Acute (short-term) toxicity to fish

An acute toxicity study with *Danio rerio* was conducted by (BASF, 2000b) according to OECD 203 under static conditions. It was a limit test with nominal 100 mg/L. No vehicle was used. This concentration was analytically confirmed with a capillary gas chromatography (limit of quantification was 2 mg/L). As the maximal water solubility of the test item is far below the analytical limit of quantification, no test substance was detected. Although the analytically confirmation was not possible because the solubility of the substance was below the limit of detection, the test was evaluated in 2001 as valid. The test temperature was 23 °C, the pH value between 8.3 and 8.4 and the dissolved oxygen was 8.2 to 8.6 mg/L. The test was valid and plausible. There were no hints for toxicity of the test substance to fish after 96 hours of testing up to its maximal water solubility concentration.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

(BASF, 2000a) conducted also an acute toxicity test to the aquatic invertebrate *Daphnia magna* according to OECD 202. They used no vehicle and a static test design. The test substance concentration was not analytically confirmed because the detection limit of the analytical method was beyond the water solubility of the test substance. Although the analytically confirmation was not possible because the solubility of the substance was below the limit of detection, the test was evaluated in 2001 as valid. The nominal test concentrations were 0, 12.5, 25, 50, and 100 mg/L. The test temperature averaged from 20.1 to 20.4 °C, the pH values were 8.0 to 8.1 and the dissolved oxygen 8.2 to 8.7 mg/L. Five organisms per vessel and 4 vessels per concentration were used with a biomass loading rate of 0.5 animals per mL. The photoperiod was 16 hours light per day with diffuse light (2-7 µE/m²s at a wave length of 400 to 700 nm). The test was valid and plausible. No acute toxicity to *Daphnia magna* occurred within 48 hours up to the limit of water solubility of the test substance.

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

(BASF, 2001a) conducted an algae test with the species *Desmodesmus subspicatus* according to OECD 201 under static conditions. No vehicle was used. The test concentrations were not analytically confirmed. Although the analytically confirmation was not possible because the solubility of the substance was below the limit of detection (see fish and daphnia acute toxicity test), the test was evaluated in 2001 as valid. The test temperature varied between 21 and 25 °C and the pH value between 7.7 and 8.4. The nominal test concentrations were 3.13, 6.25, 12.5, 25, 50, and 100 mg/L. The effects were measured via chlorophyll-a-fluorescence measurement (pulsed excitation with light flashes having a wavelength of 435 nm). The test was valid and plausible. The test substance showed no toxicity to algae within 72 hours up to the limit of water solubility. The basis of the effect was growth rate.

11.5 Long-term aquatic hazard

Table 9: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 210	*Pimephales promelas*	CAS 302776-68-7	34d-NOEC ≥ 8.8 µg/L (mean measured)	Rel. 1 GLP-study	(BASF, 2013)
OECD 211	*Daphnia magna*	CAS 302776-68-7	21d-NOEC ≥ 14.2 µg/L (mean measured)	Rel. 1 GLP-study	(BASF, 2009)
OECD 211	*Daphnia magna*	CAS 302776-68-7	21d-$NOEC_{Reproduction}$ = 0.1 µg/L (mean measured)	Rel. 1 (registrant rel. 3) GLP-study	(BASF, 2007)
OECD 201	*Desmodesmus subspicatus*	CAS 302776-68-7	72h-NOE_rC ≥ 100 mg/L (nominal)	Rel. 2 (registrant rel. 1) GLP-study	(BASF, 2001a)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.5.1 Chronic toxicity to fish

(BASF, 2013) conducted an early life stage test according to OECD 210 with the test species *Pimephales promelas* under flow-through conditions (9 L/h). The embryos were less than 3 hours old. The limit test concentration was analytically confirmed (LOQ = 2 µg/L). The measured concentrations over the first 21 d of exposure, ranged from 10.0 to 18.0 µg/L. From day 28 to day 34 of exposure, concentrations ranged from 5 to 3 µg/L. A vehicle was used. The test temperature was 23.4 to 24.9 °C, the pH value 7.8 to 8.1 and the dissolved oxygen corresponded to 67 to 97 % saturation at 25 °C (5.6 to 8.1 mg/L). A photoperiod of 16 hours light at a light intensity of 116 to 196 lux existed during the 34 days of the test. 25 fertilized eggs/embryos were exposed per vessel with 4 vessels (replicates) per concentration. The test fulfils the validity criteria of OECD 210. No signs of toxicity or abnormalities were observed during the test.

11.5.2 Chronic toxicity to aquatic invertebrates

Two long-term toxicity tests to the aquatic invertebrate *Daphnia magna* are available. Both of them were conducted according to OECD 211.

(BASF, 2007) used semi-static test conditions (renewal of the test medium every 2 to 3 days) with a temperature of 19 to 20 °C, a pH value of 7.2 to 8.3 and a content of dissolved oxygen above 7.7 mg/L. The photoperiod was 16 hours light per day (60 – 120 lux). A vehicle was used (acetone). The test concentrations were analytically confirmed (LC-MS/MS-method with a limit of quantification of 2 µg/L). At the lower concentrations, the test substance was not analysed and the recovery rates for the upper two concentrations were used to extrapolate the lower ones. This results in the concentration range: 1, 3.2, 10, 32, and 100 µg/L nominal or 0.1, 0.3, 0.9, 2.2, and 12.2 µg/L mean measured. The recovery rates of the two highest test concentrations declined despite the renewal of the test medium every 2 to 3 days. The registrant commented that there is the possibility that also not dissolved testing material was analysed using acetonitrile in sample preparation before analysing it. In this respect there are no hints in the test report. One organism per vessel and ten vessels per concentration were used in the test. The registrant assessed the test with reliability 3 amongst others (see below) because they question "to what extend dissolved and/or undissolved test substance was present in the test and if any potential outcome of the study might be related to substance intrinsic properties or rather is due to physical interactions with the material". It is possible, that the effects occurred due to physical interactions but as there were no remarkable observations on the behaviour of the test item in the test water concerning e.g. turbidity or inhomogeneous dispersion, it cannot be excluded that the test material caused the effects and so we do not share this appraisal. In the study report, the effects of the test substance on reproduction were compared to historical control of *Daphnia magna* as the reproduction rates appeared "unusual high" to the authors. Therefore, the report concludes that there are no effects from the test substance on the test organisms. According to OECD 211, the results from the exposed *Daphnia magna* are compared to the control in the test in order to determine the LOEC and NOEC. Additionally there is only a validity criterion for a minimal reproduction rate (mean number of live offspring produced per

parent animal surviving at the end of the test is ≥ 60) and not for a maximum. Taking the control from the test into account, the 21 day LOEC for reproduction is 0.3 µg/L and therefore the NOEC is 0.1 µg/L (ToxRat version 2.09; Williams t-test procedure). The test fulfils the validity criteria of OECD 211. As required by the OECD Guidance 211 only the live brood of the surviving adults was taken into account. The registrant notes that in up to five replicates per concentration the upcoming hatch was not taken into account (especially at the highest test concentration), which may have required an extension of the study period. The test was not extended. Analysing all offspring from the third hatch, irrespective on which exact day this occurred, results in the same NOEC of 0.1 µg/L based on mean measured concentrations or 1 µg/L based on nominal concentrations as using the data for day 21.

(BASF, 2009) used an analytical confirmation (extraction with n-hexane, GC-MS on nonpolar stationary phase, quantification with internal standard). For the preparation of the medium, a saturated solution of the test substance in the dilution water was prepared using a saturation column. This means that the test substance was dissolved in acetone and poured over glass wool in a stainless steel pan and acetone was evaporated. The glass wool with the attached test substance was packed into a glass column. Below the glass wool a cellulose plug was situated to keep particulate material in the column. The packed column was rinsed with demineralized water and afterwards with M4 medium. To generate the saturated test solution, after 4 days M4 medium was pumped circularly through the saturation column for one day. The mean measured concentration in the stock solution was 14.3 µg/L (8 – 30 µg/L) and in the test solution 14.2 µg/L (5 – 31 µg/L). The test was a limit test with flow-through conditions and a test temperature of 19 to 21 °C, a pH value of 8.0 to 8.2, and a content of dissolved oxygen of 8.4 to 8.9 mg/L. The photoperiod consisted of 16 hours light per day (680 – 741 lux at a wave length of 400 – 750 nm). Five organisms per vessel and four vessels per concentration were used. The test fulfils the validity criteria of OECD 211. No effects occurred up to 14.2 µg/L (mean measured).

As the most protective valid result is a NOEC for reproduction of 0.1 µg/L (mean measured) or 1 µg/L (nominal) from (BASF, 2007). This result will be used for classification.

11.5.3 Chronic toxicity to algae or other aquatic plants

(BASF, 2001a) conducted an algae test with the species *Desmodesmus subspicatus* according to OECD 201 under static conditions. No vehicle was used. The test concentrations were not analytically confirmed. Although the analytically confirmation was not possible because the solubility of the substance was below the limit of detection (see acute fish and daphnia toxicity test), the test was evaluated in 2001 as valid. The test temperature varied between 21 and 25 °C and the pH value between 7.7 and 8.4. The nominal test concentrations were 3.13, 6.25, 12.5, 25, 50, and 100 mg/L. The effects were measured via chlorophyll-a-fluorescence measurement (pulsed excitation with light flashes having a wavelength of 435 nm). The test was valid and plausible. The test substance showed no toxicity to algae within 72 hours up to the limit of water solubility.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

Table 10: Comparison with criteria for acute aquatic hazards

	Criteria for environmental hazards	**Uvinul A Plus**	**Conclusion**
Acute Aquatic Toxicity	Cat. 1: $LC_{50}/EC_{50}/ErC_{50} \leq 1$ mg/L	Fish: 96h-LC_{50} > 100 mg/L (nominal) Invertebrates: 48h-EC_{50} > 100 mg/L (nominal) Algae: 72h-E_rC_{50} > 100 mg/L (nominal)	No acute aquatic toxicity up to the limit of water solubility

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

Table 11: Comparison with criteria for long-term aquatic hazards

	Criteria for environmental hazards	Uvinul A Plus	Conclusion
Rapid Degradation	Half-life hydrolysis < 16 days Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % ThCO2, ThOD)	Half-life hydrolysis > 16 days (estimated) 0-10 % after 28 days (O_2 consumption) => not readily biodegradable	**Not rapidly degradable**
Bioaccumulation	BCF > 500 or log Kow ≥ 4	BCF < 360 (lipid and growth corrected)	**Not bioaccumulative**
Aquatic Toxicity	Non-rapidly degradable substances: Based on long-term toxicity data: Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L	Fish: 34d-NOEC ≥ 8.8 µg/L (mean measured) Invertebrates: 21d-$NOEC_{Reproduction}$ = 0.1 µg/L (mean measured) Algae: 72 h-NOE_rC ≥ 100 mg/L (nominal)	**Aquatic chronic 1, H410, M= 1000** (based on *Daphnia magna* $NOEC_{reproduction}$ = 0.0001 mg/L)

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Uvinul A Plus is not rapidly degradable and the most protective valid long-term toxicity no effect concentration is 0.0001 mg/L. This results in a classification of Uvinul A Plus as Aquatic Chronic 1 (M-factor of 1000) and a labelling with H410.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate is used in cosmetics and personal care products. The substance is currently listed in Annex VI of the CLP Regulation (EC) 1272/2008 with a classification for environmental hazards as Aquatic Chronic 4 – H413. The Dossier Submitter (DS) proposed to classify the substance as Aquatic Chronic 1 – H410 (M=1000) based on lack of rapid degradation and a 21 days mean measured NOEC value of 0.0001 mg/L for *Daphnia magna*.

Degradation

There was one ready biodegradability test available on the substance (OECD TG 310 F, GLP) using 30 mg/L inoculum (domestic activated sludge, non-adapted) and 100 mg/L test substance (BASF, 2001b). The test was performed at pH 7.3-7.4. After 28 days, 2–5 % O_2 consumption was observed indicating that hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate is not readily biodegradable. The percentage degradation of the reference substance (aniline) has reached the pass level after 14 days (80-90%).

The hydrolysis of the substance was estimated by EPI Suite HYDROWIN (v2.00). At pH 8, the half-life was predicted to be 250 days and at pH 7 6.9 years.

The photochemical degradation in air was investigated using the SRC AOP v1.92, 2007 estimation tool. A rate constant of 0.0000000002252403 cm³/molecule*sec and a half-life in the atmosphere of 1.7 hours was calculated assuming a 24 hours day and an OH-radical concentration of 5.0E+05 molecules/cm³. Hence, if the substance will be exposed to air, it will be rapidly degraded by photochemical degradation. Nevertheless, based on estimated Henry´s law constant of 0.000019 Pa·m³/mol it will not evaporate from water surface to air.

The DS considered hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate as not rapidly degradable for classification purposes.

Bioaccumulation

The octanol-water partition coefficient (log K_{OW}) of 6.2 at 24°C (without adjustment of pH value) was measured by EU Method A.8 (HPLC method).

A fish bioaccumulation study (OECD TG 305, GLP) is also available. The zebrafish (*Danio rerio)* was exposed to two nominal concentrations (0.1 and 1 µg/L) of the ^{14}C-radiolabelled test substance for 28 days in a flow-through system, followed by a 16 days (1 µg/L) and 21 days (0.1 µg/L) depuration period. The concentration in the fish was found to reach steady state within 7 days for both concentration groups. A steady-state BCF of 126.8 L/kg (0.1 µg/L) and 215.4 L/kg (1 µg/L) and a kinetic BCF of 120.3 L/kg (0.1 µg/L) and 204.6 L/kg (1 µg/L) were reported. During the depuration phase the half-life time for the test substance in fish was 0.9 days (0.1 µg/L) and 1.4 days (1 µg/L). Approximately 90 % of the steady state-concentration of the test substance was excreted after 3.1 days (0.1 µg/L) and 4.8 days (1 µg/L). The lipid content in the study was in the range between 3.01 and 4.62% over the whole uptake and elimination period. Llipid and growth corrected BCFs were 360 L/kg for the higher exposure concentration (1 µg/L) and 230 for the lower exposure concentration (0.1 µg/L).

The result of the above study is supported by a screening study according to OECD TG 305. The zebrafish (*Danio rerio)* were exposed to a single nominal concentration (1 µg/L) of the ^{14}C-radiolabelled test substance for 21 days in a flow-through system, followed by a 7-days depuration period and the time to steady state was approximately 1 day. A steady-state BCF of 193.44 L/kg and kinetic BCF of 225.6 L/kg was reported. During the depuration phase the half-life of the test substance in fish was 1.17 days (DT90 = 3.9 days).

The DS considered hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate as a substance with low potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following Table (the key endpoints used in hazard classification are highlighted in bold). hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate has been shown to be poorly water soluble (16 µg/L at 20°C).

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

Method/Exposure	Test organism	Endpoint	Toxicity values in mg a.s./L	Reference/Remarks (reliability refers to Klimisch scores)
Short-term toxicity				
OECD TG 203 Static	*Danio rerio*	96-h LC_{50}	>100 nom	(BASF, 2000b) Rel. 2
OECD TG 202 Static	*Daphnia magna*	48-h EC_{50}	>100 nom	(BASF, 2000a) Rel. 2
OECD TG 201 Static	*Desmodesmus subspicatus*	72-h E_rC_{50}	>100 nom	(BASF, 2001a) Rel. 2
Long-term toxicity				
OECD TG 210 Flow through	*Pimephales promelas*	36-d NOEC	>0.0088 mm	(BASF, 2013) Rel. 1
OECD TG 211 Flow through	*Daphnia magna*	21-d NOEC	≥0.0142 mm	(BASF, 2009) Rel. 1
OECD TG 211 Semi-static	*Daphnia magna*	21-d NOEC	**0.0001** mm	(BASF, 2007)* Rel. 1 (DS), Rel.3 (REACH registrant)
OECD TG 201 Static	*Desmodesmus subspicatus*	72-h NOE_rC	>100 nom	(BASF, 2001a) Rel. 2
*Study is considered reliable by DS but unreliable by REACH registrant. mm = mean measured; nom = nominal;				

Acute toxicity

Acute aquatic toxicity data are available for fish, invertebrates and algae. The DS proposed not to classify hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate as acutely hazardous to the aquatic environment on the basis that the short-term (acute) aquatic ecotoxicity test results showed no toxic effects to aquatic organisms (algae, daphnia and fish) at concentrations up to the water solubility limit.

Chronic toxicity

Long-term aquatic toxicity data are available for fish, invertebrates and algae.

The limit test on early life-stage toxicity of the test substance to embryos, larvae and young fish was examined according to OECD TG 210 with the fish *Pimephales promelas* in a flow through test system set-up (BASF, 2013). No chronic toxicity to fish was observed up to the limit of water solubility under test conditions (8.8 µg/L).

Two chronic toxicity studies with *Daphnia magna* preformed according to OECD TG 211 were reported by the DS. In the first study (BASF, 2007) the 21 days NOEC based on reproduction was 0.0001 mg/L (mean measured) or 0.001 mg/L (nominal). The study was considered valid by the DS but unreliable by the REACH Registrant, as some validity criteria with respect to

shortcomings in the test performance (solvent control, test media) were not (see public consultation). In the second study (limit test) no chronic toxicity to *Daphnia magna* was observed up the limit of water solubility (14.2 µg/L) (BASF, 2009).

A static algal toxicity test according to OECD TG 201 was performed on *Desmodesmus subspicatus* (BASF, 2001a). The test substance showed no toxicity to algae within 72 hours up to the limit of water solubility.

The chronic aquatic classification proposed by the DS (Aquatic Chronic 1, M=1000) was based on the, in their opinion reliable, BASF (2007) chronic toxicity study on *Daphnia magna*.

Comments received during public consultation

Four Member State Competent Authorities (MSCAs) and one company-manufacturer submitted comments during public consultation. One MSCA supported no classification for aquatic acute hazards. Three commenting MSCAs supported the DS proposal to modify the classification to Aquatic Chronic 1, M-factor=1000, while one MSCA did not express a view in relation to the chronic classification.

One MSCA in the first comment pointed out that the substance has a low water solubility (0.01 mg/L) and with a log Koc of 5.1 one might would expect adsorption to organic matter. However, this MSCA agreed with the DS that it cannot be excluded that the observed effects in the BASF 2007 study were due to exposure because no physical effects on the test organisms by non-dissolved test material were reported in the study. In the following targeted public consultation, the DS came to the conclusion that it is possible that the effects occurred due to the particles (physical effect).

The second comment refered to the use of historical control data by the REACH Registrant in the BASF 2007. The MSCA agreed with DS that test results should be compared to the control data of the study because the same study conditions are applied for control and test concentrations. According to OECD TG 211, data from treated animals should be compared with concurrent study control data. RAC agrees with the DS and the commenting MSCA.

The same MSCA considered both chronic studies on invertebrates (BASF, 2007 and BASF, 2009) valid.

A second commenting MSCA required further data to determine the NOEC reliability in the key study (BASF, 2007) and further information regarding QSAR predictions that is available in the REACH registration dossier, available at the ECHA dissemination website.

The company-manufacturer disagreed with the DS proposal to modify the classification to Aquatic Chronic 1, M-factor=1000. The company was of the opinion that the DS proposal is based on a misinterpretation of a chronic daphnia toxicity study (BASF, 2007), which is considered invalid according to the OECD TG 211 by the company due to shortcomings in the test performance (no adequate solvent control used and nutrition composition of the M4 media of control group differed from the treatment groups). The company submitted along with the comments also two expert statements (Galloway, 2017; IBACON, 2017) providing further argumentation regarding the invalidity of the study together with a justification for no classification for chronic aquatic hazards. The ECHA Secretariat has also received a position paper from a Brussels Law Firm (sent on behalf of their client) to which the DS provided his response.

- No adequate solvent control used in BASF 2007 study

Regarding the missing of adequate (solvent) control in the BASF 2007 study the DS agreed with the company that according to OECD TG 211 a solvent control has to be used, when a solvent is used for the preparation of the test concentrations. The DS pointed out that in both studies, BASF 2007 and BASF 2009, the solvent (acetone) was completely evaporated before the test media was added. Therefore, it is not expected that any solvent was present in the medium during the test. Consequently, the available control group is considered an adequate reference to be compared with the treatment groups and the absence of a solvent control does not render the study unreliable. RAC agrees with the explanation and response provided by the DS.

- Differences in the preparation of the test media led to differences in the nutrition composition of the M4-control group compared to the treatment groups.

In the second amendment to the study report it is stated that *"after 2 to 3 days of stirring, precipitation was observed either floating on the surface or being stuck to the magnetic stirrer. This observation only occurred in the test concentrations and not in the control. The precipitation was not determined analytically, but identified by the laboratory assistant as iron. Therefore, it can be concluded that the test media composition was different for the daphnia of the test concentration compared to the test medium for the control group."* The DS pointed out that this observation was not described in the initial study report. Furthermore, in the expert statement by Galloway (2017) it is described that precipitation of iron was noted in the raw data report. RAC notes that the reported deviations regarding test media (precipitation of iron) are not consistent. Regarding the precipitation of iron, the DS is of the opinion that due to the fact that no analytical proof for this hypothesis was provided, this remains speculative. In DS view the reported precipitation, together with the hypothesis of the nature of the precipitate and the contradictions in the reporting are not sufficient to raise reasonable doubt about the results of the study and to consider it unreliable. RAC has no reliable information regarding the identity of the precipitate in test media. RAC is of the opinion that due to the lack of an analysis report demonstrating the presence of the iron in the test media, such a statement cannot be considered scientifically valid.

In the expert statement provided by Galloway (2017), it is stated that variations in metal concentration, including iron, can affect growth and reproduction in daphnia species (Biesinger and Christensen, 1972, Bosnir et al., 2013, Hudson et al., 2016). The DS provided an assessment of the cited publications during the second public consultation (see next section).

QSAR calculations using ECOSAR v1.00 were provided during consultation (BASF, 2018). The resulting values and explanation of the results are provided in the following Table.

***Table**: Results of the QSAR calculations*

Chronic fish toxicity		
Esters	chronic value (33 d) = 4 µg/L	There is an apparent chronic toxicity towards fish within the limit of water solubility (16 ± 3 µg/L).
Phenols	chronic value (30 d) = 8 µg/L	
Neutral organic SAR	chronic value = 4 µg/L	
Chronic daphnia toxicity		
Esters	chronic value (21 d) = 31 µg/L	No chronic toxicity towards daphnia within the limit of water solubility (16±3 µg/L).

Phenols	chronic value (21 d) = 10 µg/L	There is an apparent toxicity within the limit of water solubility.
Neutral organic SAR	chronic value = 9 µg/L	
Note: RAC considers that the QSAR predictions as presented by the company are not well documented and justified (*i.e.* no detailed assessment of applicability domain and reliability).		

After the end of the public consultation (in September 2018), new data was provided including additional experimental studies on different daphnia strains, as well as analytical investigations to identify the nature of the precipitate observed in the BASF 2007 study. This was approached by repeating the preparation of the test media according to the BASF 2007 study protocol and subsequent identification of the precipitate with appropriate analytical methods. More specifically, new *Daphnia magna* reproduction tests (OECD TG 211) performed with M4 medium with and without Fe (II), two new *Daphnia magna* reproduction tests (OECD TG 211) on different strains (including the same one as in the BASF 2007 study) and an investigation of the solubility of the test substances in M4 medium have been provided.

As a result, a second public consultation was launched on the above new information, with three MSCAs and one company-manufacturer submittinged comments..

One MSCA (the DS) provided an assessment of the additional data submitted.

Based on all available information, another MSCA considered that there are uncertainties regarding the BASF (2007) chronic toxicity to *Daphnia magna* study endpoints which impact the study reliability. The same MSCA agreed that the three valid chronic toxicity to *Daphnia magna* studies demonstrate no effects to the limit of solubility in test media.

A summary of the new information and studies submitted by Industry (June 2018) is presented in the Background document.

Additional key elements

In this section the key information from new data and new studies submitted by Industry (June 2018) is presented.

Additional analytical investigations on the identification of precipitate within the BASF 2007 study

Effect of stirring on M4 media composition

In the BASF (2007) study all test concentrations were stirred for 2 to 3 days (afterwards the precipitation was observed) but not the control group. Two analysis reports (18N01168 and 18A01078) and supplemental report for 18N01168 and 18A01078 (detailed analytical procedure) were submitted by industry (September 2018).
M4 medium was stirred under different conditions to evaluate the effect of stirring on the nutrient composition (*i.e.* iron concentration). Variation in stirring time, temperature, stirring velocity as well as the presence of the test substance up to the water saturation concentration had been tested for effects on the metal content. The total content of the specified elements in the solution were determined by ICP-MS. The analytical method (ICP-MS) did not allow to distinguish between different species such as Fe^{2+} and Fe^{3+}. The results of the analytical investigations are presented in the following Tables.

Table: *Measured metal concentrations within analysis report (18N01168)*

Sample description	**Conc. Cu (mg/L)**	**Conc. Fe (mg/L)**	**Conc. Mn (mg/L)**	**Conc. Mo (mg/L)**	**Conc. Zn (mg/L)**
M4 medium without stirring, 25°C, 66h	0.03	0.12	0.10	0.03	< 0.03
M4 medium, with stirring (250 rpm), 25°C, 66h	0.03	0.06	0.10	0.03	< 0.03
M4 medium, with stirring (250 rpm), 30°C,66h	0.03	0.06	0.09	0.03	< 0.03
M4 medium (saturated), with stirring (250 rpm), 25°C, 40h	0.03	0.06	0.10	0.03	< 0.03
M4 medium (saturated), with stirring (250 rpm), 30°C, 40h	0.03	0.05	0.09	0.03	< 0.03

Table: Measured metal concentrations within analysis report (18A01078)

Sample description	**Conc. Cu (mg/L)**	**Conc. Fe (mg/L)**	**Conc. Mn (mg/L)**	**Conc. Mo (mg/L)**	**Conc. Zn (mg/L)**
M4 medium without stirring, 25°C, 48h	0.01	0.17	0.10	0.03	n.a.
M4 medium with slow stirring (100 rpm), 25°C, 48h	0.01	0.13	0.10	0.03	n.a.
M4 medium with moderate stirring (250 rpm), 25°C, 48h	0.01	0.13	0.10	0.03	n.a.
M4 medium with fast stirring (100 rpm), 25°C, 48h	0.01	0.13	0.10	0.03	n.a.
M4 medium without stirring, 25°C, 96h	0.01	0.17	0.09	0.03	n.a.
M4 medium with slow stirring (100 rpm), 25°C, 96h	0.01	0.12	0.10	0.03	n.a.
M4 medium with moderate stirring (250 rpm), 25°C, 96h	0.01	0.11	0.10	0.03	n.a.
M4 medium with fast stirring (500 rpm), 25°C, 96h	0.01	0.12	0.07	0.03	n.a.

The results of the additional analytical investigations show that:

- When the M4 medium is stirred, the iron concentration is reduced, while without stirring no change in iron concentration could be observed (0.12 to 0.06 mg/L and 0.17 to 0.13 mg/L).
- Stirring did not affect the concentration of the other elements (both Tables above).
- The addition of test substance and the variation of the temperature have no influence on the elemental concentrations.

- The variation of stirring speed has no influence on the elemental concentrations.

The DS pointed out that for both experiments there was no information on replicates and therefore the variation of the iron content within the same solution was not apparent.

Daphnia magna reproduction tests with M4 medium with and without Fe(II) (BASF 2018c)

The impact of the absence of soluble iron (Fe(II) from the M4 medium) on the reproduction of *Daphnia magna* STRAUS, clone 5 was determined under semi-static test system set-up. The study was performed according to OECD TG 211. Daphnids were exposed to a standard M4 medium (control group) and M4 medium without Fe(II)-EDTA-complex for 21 days. In comparison to the control group, no statistically significant effect on parent mortality, parental length, number of living offspring, number of immobile neonates, aborted eggs and time to first brood (Wilcoxon test, one-sided, not significant) was seen. However, the range of brood deposition time from the first to the last observed brood (*i.e.* the fourth brood) became much wider in the iron-free M4 medium group, compared to the M4 medium group with 4 – 6 and 2 – 3 days respectively (first Figure below). Daphnids raised in the absence of Fe(II) in the M4-medium demonstrated a delay in the average brood deposition day of the first four broods. Whereas this delay was not statistically significant after the first, second and third brood, the delay of the fourth brood was statistically significant compared to the control group (Wilcoxon test, one-sided, $p \leq 0.05$) (second Figure below). Taking the effects of iron deficiency in the medium into account, a delay in the last brood deposition could influence the overall number of offspring per female, as delayed broods might not be completely deposited within the given test duration of 21 days. The fourth brood from some individuals might be deposited on day 22 and thus after the study has terminated. This could influence the evaluation of the study results as the number of broods were not equal to the control group (3 versus 4 broods within 21 days). This can lead to a misinterpretation of the fecundity endpoint if the test is terminated on day 21.

		Day																				
Test group	Replicate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Sum
Standard M4	1												10			18	dead	dead	dead	dead	dead	dead	
	2									13			26				39			41			119
	3											18			33			38			42		131
	4									16			28			41				41			126
	5									10			19			35				39			103
	6									13			16			40				41			110
	7									13			29			43				40			125
	8									9			32			43				36			120
	9											16			31			17			31		95
	10											10	1	20			39			38			108
	Replicate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Sum
M4 without Fe^{2+}-EDTA	1											12			25				40			38	115
	2									17		16			35			35			44		147
	3												29				39			38			106
	4											16			36			40			41		133
	5									13				19			41			43			116
	6											19			26			28			38		111
	7									12			29				36			39			116
	8											14			32				35			44	125
	9											20			32			35			40		127
	10											17	1		31				33			45	127

1st brood
2nd brood
3rd brood
4th brood
5th brood

- No difference in the number of offspring/clutch after 21 days
- **Greater variability** clutch deposition day in daphnids without Fe^{2+} (4 – 6 d versus 2 – 3 d in control)
- All M4-daphnids completed 4th clutch at day 20, whereas „Fe^{2+}-free" daphnids needed another day (i.e. day 21), and in one case even more (> 21 d)

Figure. Individual days of clutch deposition of daphnids from full M4 medium and iron free M4 medium (Declaration on additional data)

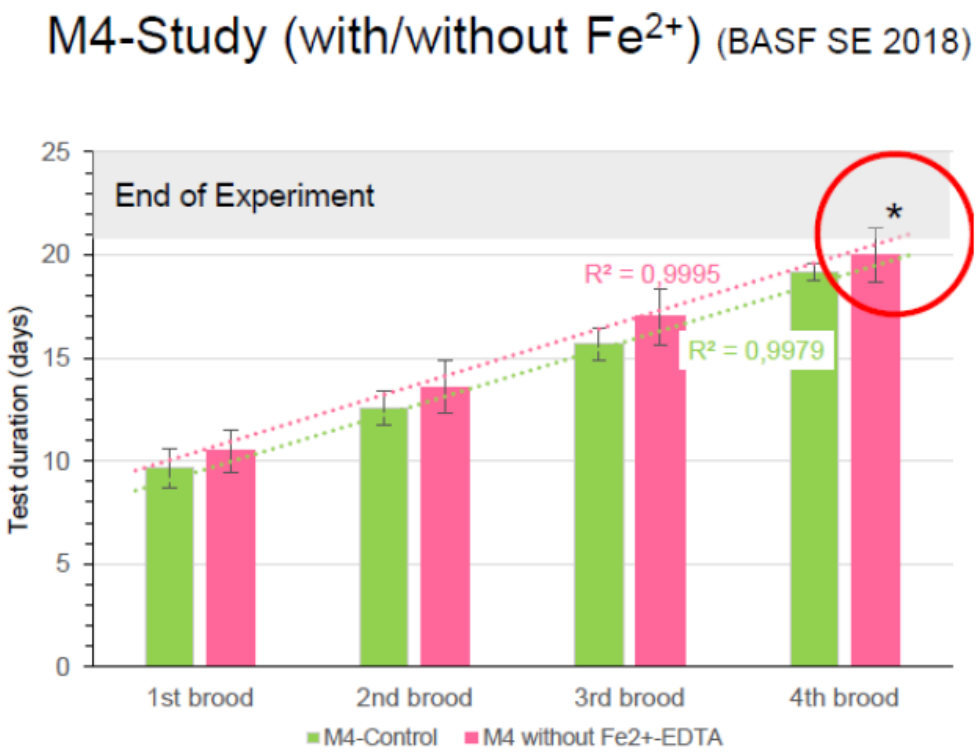


Figure. Average day of brood deposition from brood 1 to 4 during 21 days of exposure to M4 and iron free M4 medium (*Statistically significant different from control, Wilcoxon test (one-sided, $p \leq 0.0311$)) (Declaration on additional data)

In the view of the industry these observations are very much in line with the findings within the BASF (2007) study, in which a similar delay on the average day of clutch deposition was observed, especially towards the end of the experiment: the control group in that study needed 20 days at average to lay the fourth clutch, the treatment groups needed at average almost 21 days. Also, some of the adult daphnids did not deliver a fourth clutch although they have shown a proper reproduction during the three previous clutches. It is expected that the offspring from the fourth missing clutch would have been available by day 22, if the duration of the study would have been extended for another day. RAC notes that there is no statistical significant difference in average day of clutch deposition between the control group and the highest treatment group in the BASF study (2007).

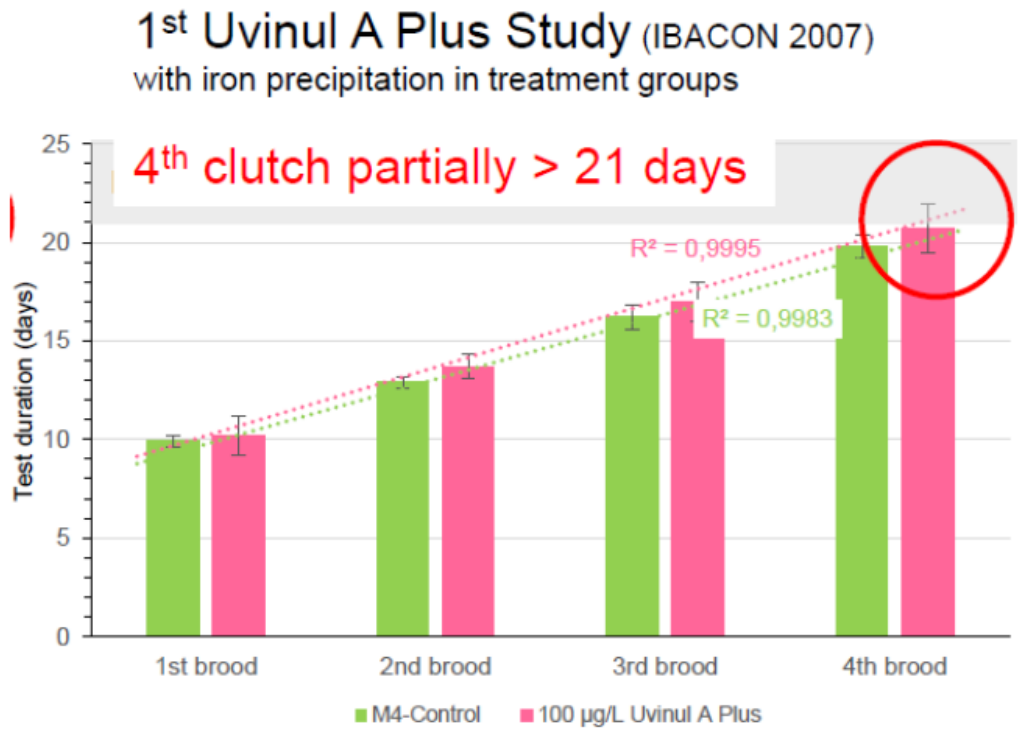


Figure. BASF 2007 Comparison on the average day of clutch deposition of the highest treatment group, compared to the M4 medium (Declaration on additional data).

		Day																				
Test group	**Replicate**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
M4-Control	1	0	0	0	0	0	0	0	0	8	0	0	31	0	0	34	0	0	43	0	0	0	**116**
	2	0	0	0	0	0	0	0	0	0	20	0	0	25	0	0	45	0	0	0	45	0	**135**
	3	0	0	0	0	0	0	0	0	0	20	0	0	33	0	0	44	0	0	0	37	0	**134**
	4	0	0	0	0	0	0	0	0	0	13	0	0	37	0	0	41	0	0	0	33	0	**124**
	5	0	0	0	0	0	0	0	0	0	19	0	0	31	0	0	48	0	0	0	39	0	**137**
	6	0	0	0	0	0	0	0	0	0	15	0	0	24	0	0	0	46	0	0	51	0	**136**
	7	0	0	0	0	0	0	0	0	0	17	0	0	35	0	0	0	52	0	0	49	0	**153**
	8	0	0	0	0	0	0	0	0	0	14	0	0	34	0	0	37	0	0	0	42	0	**127**
	9	0	0	0	0	0	0	0	0	0	14	0	0	25	0	0	0	50	0	0	44	0	**133**
	10	0	0	0	0	0	0	0	0	0	14	0	0	39	0	0	18	0	0	0	35	0	**106**
100 µg/L	**Replicate**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Sum**
(nominal)	1	0	0	0	0	0	0	0	0	0	0	17	0	0	40	0	0	0	48	0	0	48	**153**
Highest Test	2	0	0	0	0	0	0	0	0	0	0	13	0	0	13	0	0	0	42	0	0	0	**68**
concentratio	3	0	0	0	0	0	0	0	0	0	0	18	0	0	29	0	0	0	39	0	0	0	**86**
n	4	0	0	0	0	0	0	0	0	11	0	0	0	25	0	0	33	0	0	0	33	0	**102**
	5	0	0	0	0	0	0	0	0	13	0	0	0	28	0	0	41	0	0	0	36	0	**118**
	6	0	0	0	0	0	0	0	0	12	0	0	0	25	0	0	34	0	0	0	29	0	**100**
	7	0	0	0	0	0	0	0	0	0	0	22	0	0	14	0	11	0	30	0	0	0	**77**
	8	0	0	0	0	0	0	0	0	0	0	15	0	0	25	0	0	0	32	0	0	0	**72**
	9	0	0	0	0	0	0	0	0	9	0	0	0	14	0	0	42	0	0	0	36	0	**101**
	10	0	0	0	0	0	0	0	0	0	0	26	0	0	0	9	0	0	30	0	0	0	**65**

1st brood
2nd brood
3rd brood
4th brood

- **Greater variability in the days of clutch deposition** (3 - 4 d* versus 2 – 3 d in control)* among adult daphnids, lead to **missing 4th clutch** in the treatment groups

Figure. Individual days of clutch deposition of daphnids (highest nominal concentration) exposed daphnids compared to the M4 medium. (*2 – 5 days in the lower treatment groups) (Declaration on additional data).

The degree of "iron" precipitation within the BASF (2007) study was not quantified but observed visually in several treatment groups over more or less the entire duration of the study (preparation of test media for the treatment groups) and thus explains the variability on the day of the fourth clutch deposition among the treatment groups and the absence of a concentration depended substance related effect (Figure below). For the treatment groups, the error bar of the statistical evaluation for the fourth clutch extends the 21 days duration of the chronic daphnia test.

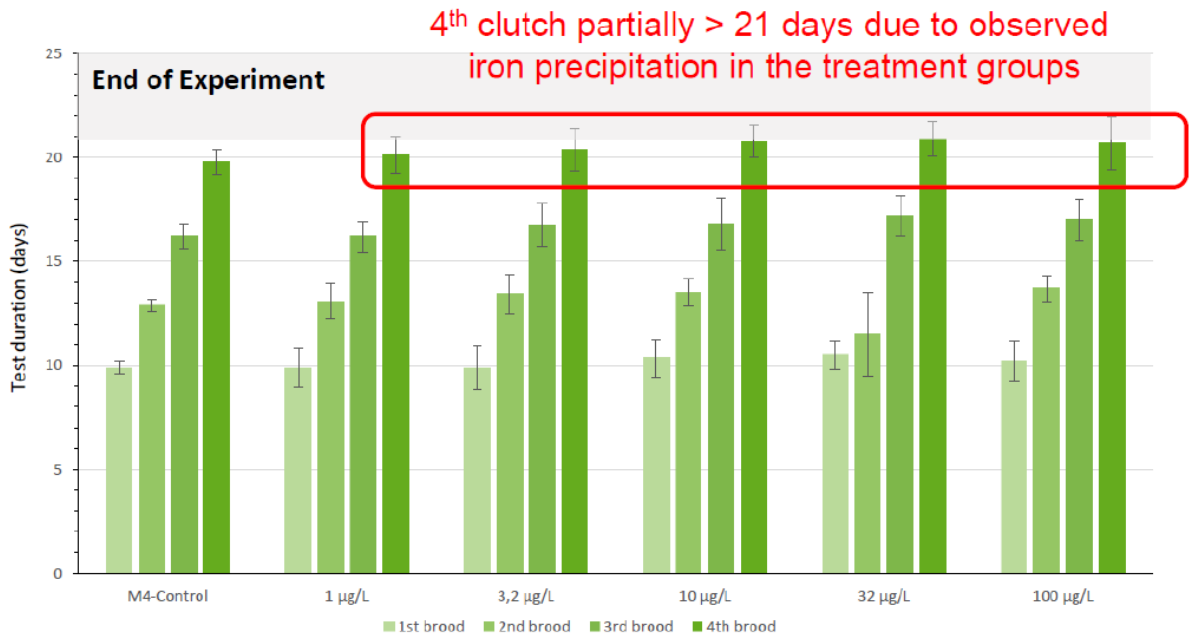


Figure. IBACON (2007): Average day of clutch deposition (Declaration on additional data)

Additionally, information from two publications were provided. These two publications were already quoted by the DS in RCOM document. A publication from Hudson *et al.* (2016) showed that daphnids started reproduction significantly later, when they are fed with algae containing lower contents or no Fe(II). As is depicted in the Figure below from the same publication, the time to first reproduction shifted from 8-9 days to roughly 11 days, when the feed algae were completely depleted from iron. The curve shows a clear dose-response correlation.

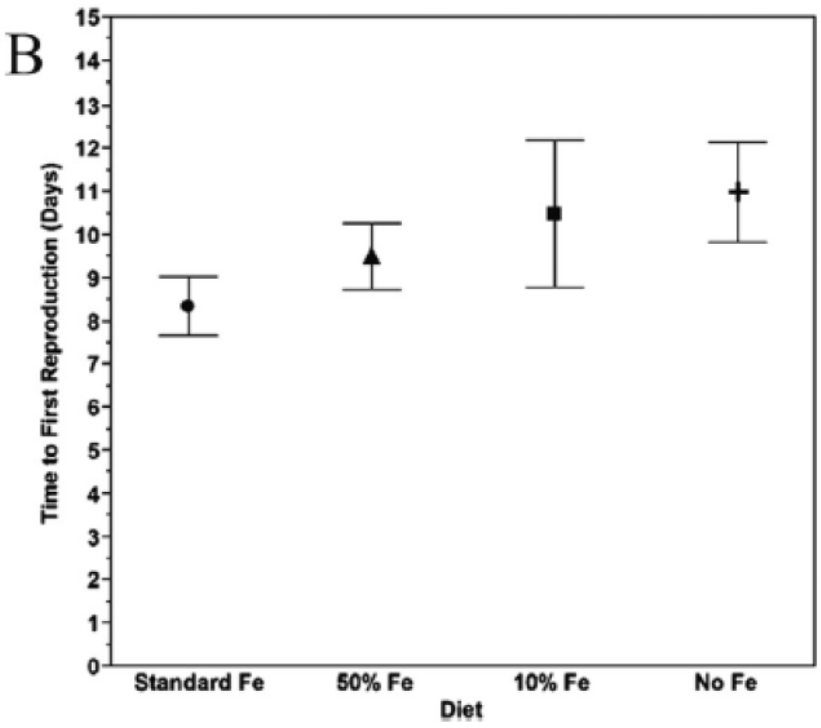


Figure. Hudson *et al*. (2016): Time to first reproduction increases with lower iron concentrations in the feed (algae)

Also, Dave (1984) could show significant effects of the Fe (II) concentration in the medium on the reproduction rate of female daphnids as is demonstrated in the Figure below.

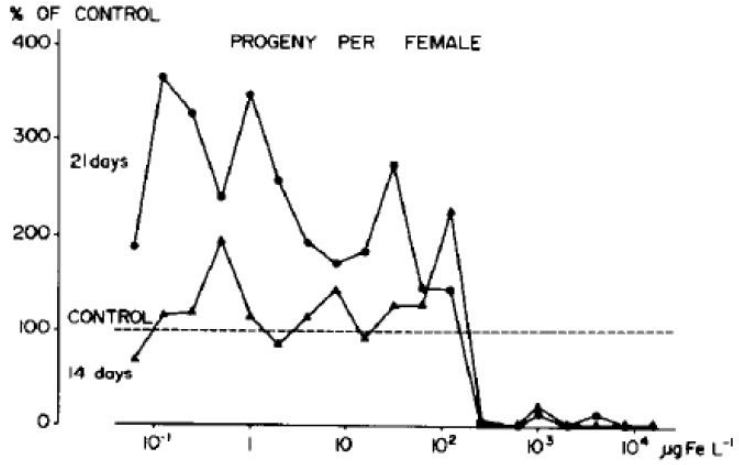


Fig. 3. Effect of waterborne iron on reproduction in *Daphnia* after 14 and 21 days.

Figure. Dave (1984): Stimulation of daphnid reproduction at low iron concentrations

Two new OECD TG 211 *Daphnia magna* Reproduction Tests

Two new *Daphnia magna* reproduction tests (OECD TG 211) by BASF (2018) were made available to RAC. The aim of the studies was to assess the effects of the substance on *Daphnia magna* over 21 days and to address any differences in sensitivity of various daphnia strains (including the one from the BASF 2007 study). The studies were performed on different strains/clones, *Daphnia magna* STRAUS (clone M10) (BASF 2018a) and *Daphnia magna* STRAUS (clone 5) (BASF 2018b).

Both studies were limit tests with semi-static test conditions (renewal of the test medium every 24 h) with a temperature, pH and a content of dissolved oxygen within acceptable guideline specifications. The photoperiod consisted of 16 hours light per day (679 – 752 lux (BASF 2018a) and 698 – 760 lux (BASF 2018b) at a wave length of 400 – 750 nm). hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate is poorly soluble in water. Therefore a saturated stock solution was prepared using a saturation column. The test substance was dissolved in acetone and poured over glass wool in a stainless steel pan and acetone was completely evaporated. The glass wool with the attached test substance was packed into a glass column. A cellulose plug below the glass wool was used to keep particulate material in the column. The packed column was rinsed with demineralised water for approx. 48 h and afterwards with M4 medium for approx. 2 days. To generate the saturated test solution, M4 medium was recirculated through the saturation column for at least one day before first use. A blank control column was identically prepared without test substance and used to treat the test medium for the control group. Each treatment group consisted of 10 replicates with one daphnid each (individual exposure). Both studies fulfil the validity criteria of OECD TG 211.

In the first study (BASF 2018a) the measured concentrations at the start of each renewal interval (initial concentrations) were between 68% and 133% of the reported water solubility value (16 µg/L) and the overall mean initial measured was 88% of the water solubility. At the end of the 24-h renewal interval concentrations generally decreased and were in the range between 32 % and 108 % of the corresponding initial measured concentrations in biotic samples with the a mean of 84 % from the mean initial value. Since concentrations generally varied by more than ±20% over the renewal interval, a time-weighted mean (TWM) was calculated to evaluated the test results. The time weighted mean was 91% of the mean initial measured concentration. No significant mortality, reduced reproduction or any other additional significant adverse biological effects or abnormal behaviour were observed in any of the test treatments. The 21-day NOEC based on time-weighted mean measured concentrations was ≥ 12.7 µg/L.

In the second study (BASF 2018b) the measured concentrations at the start of each renewal interval (initial concentrations) were between 73 % and 99 % of the reported water solubility value (16 µg/L) and the overall mean initial measured was 88 % of the water solubility. At the end of the 24-h renewal interval concentrations generally decreased and were in the range between 36 % and 119 % of the corresponding initial measured concentrations in biotic samples with the a mean of 67 % from the mean initial value. Since concentrations generally varied by more than ±20% over the renewal interval, a time-weighted mean (TWM) was calculated to evaluated the test results. The time weighted mean (TWM) was 81% of the mean initial measured concentration. No significant mortality, reduced reproduction or any other additional significant adverse biological effects or abnormal behaviour were observed in any of

the test treatments. The 21-day NOEC based on time-weighted mean measured concentrations was ≥ 11.4 µg/L.

In the BASF 2018b study the same clone 5 had been used as in BASF 2007 study (originated from IBACON). The aim of the study was to found out whether the clone used in BASF 2007 study is more sensitive than the same clone 5 used in the BASF 2009 study (originated from BASF). Comparison of the results of the of BASF 2018c (NOEC ≥ 11.4 µg/L) and BASF 2009 study (NOEC ≥ 14.2 µg/L) revealed that the Daphnia strain from IBACON is not more sensitive in comparison to the one from BASF. The results of the second study (BASF 2018a) in which the clone M10 (supplied from ECT Ökotoxikologie facility) was used, showed that the M10 clone (NOEC ≥ 12.7 µg/L) was not more sensitive than the one used at BASF (NOEC ≥ 14.2 µg/L) or IBACON (NOEC ≥ 11.4 µg/L).

Water solubility study (BASF 2009, 08E03159)

A water solubility study (BASF 2009, 08E03159) was provided, in which two methods were used: a) column eluate method using M4-Medium or Milli-Q-water and b) flask method using Milli-Q-water. The first method (a) using M4-Medium resulted in a maximum water solubility of 13 ± 6 µg/L at 20°C. Using Milli-Q-water in the first method resulted in a maximum water solubility of 16 ± 3 µg/L at 20°C and the second method (b) in a maximum water solubility of 25 µg/L at 20°C. The solubility of the test substance is lower in M4 medium than in pure water.

Assessment and comparison with the classification criteria

Degradation

In the absence of supporting information to justify the QSAR prediction of hydrolysis, no conclusion about the hydrolysis half-life can be drawn by RAC. The substance showed 2-5 % degradation after 28 days in the ready biodegradation test (OECD TG 310 F) and is, thus, considered to be not readily biodegradable. RAC notes that the ready biodegradation study was performed using a test substance concentration that is more than four orders of magnitude above the water solubility limit, so dissolution kinetics may be one reason for limited degradation in this study. Based on available data, RAC agrees with the DS's conclusion that available degradation information does not indicate that it is ultimately degraded (>70%) within 28 days (equivalent to a degradation half-life of <16 days). Consequently, it is considered to be not rapidly degradable for the purposes of classification under the CLP Regulation.

Bioaccumulation

RAC agrees with the DS that hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate has a low potential to bioaccumulate in aquatic organisms. The basis for this is that the measured BCF value of 360 L/kg (lipid and growth corrected) is below the decisive CLP Regulation criterion of 500.

Acute toxicity

Aquatic acute toxicity data on hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate are available for fish, invertebrates and algae. No effects on aquatic organisms were observed up to the water solubility limit. RAC supports the DS´s proposal that **no classification for acute aquatic hazards is**

warranted.

Chronic toxicity

RAC assessed the two new *Daphnia magna* reproduction tests (BASF 2018a and BASF 2018b) and considers them valid and reliable, thus, these studies should be used for classification purposes. In the view of RAC, three reliable chronic toxicity studies on invertebrate *Daphnia magna* are relevant for classification, namely BASF 2009, 2018a and 2018b. RAC considers that the weight of evidence from the by now large body of chronic aquatic data for this substance, shows that there is no chronic aquatic toxicity within the limit of its solubility in water.

21 days Daphnia magna reproduction study (BASF, 2007)

Regarding the identity of precipitate in test media, there is no contemporaneous analysis report. However, the precipitate was identified by the laboratory assistant as iron, based on its colour (brownish) and the fact that it was associated with the magnetic stirrer in the test beaker; it was thus considered to be iron(III)oxide. Additional analytical investigations at a much later date showed that stirring of the M4 medium reduces its iron content and thus impacts the final medium composition used for the *Daphnia magna* reproduction test. This means that control and treatment groups within the BASF (2007) study could have had different nutrient compositions since all test solutions were stirred for 2 to 3 days (but not the control). Iron deficiency in the M4 medium has a negative impact on the reproduction behaviour of daphnids. Fe is essential for Daphnia in haemoglobin synthesis and reproduction (Dave, 1984). Hudson *et al*. (2016) study showed a reduced (but non-significant) maturation rate in Daphnia fed reduced Fe diets. Results of the BASF (2018c) study showed that the absence of soluble iron (Fe(II) from the M4 medium) affect the reproduction of *Daphnia magna* in form of delayed deposition of the brood. A similar delay on the average day of brood deposition was observed in the BASF (2007) study. Therefore the results of the BASF (2007) study are considered by RAC not to be reliable.

Conclusion

RAC is of the opinion that adequate chronic toxicity data are available for all three trophic levels (fish, daphnia and algae). The available information shows no adverse effects to aquatic organisms at concentrations up to the water solubility limit in all reliable tests.

Because the substance is not rapidly degradable, not bioaccumulating and has a chronic toxicity with NOECs above water solubility or hreater than 1 mg/L, RAC is of the opinion that **no classification for chronic aquatic toxicity is warranted**.

12 REFERENCES

BASF (2000a): 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesaeurehexylester - Determination of the acute effect on the swimming ability of the water flea Daphnia magna Straus. 99/0765/50/1, date: 2000-07-19. Department of Experimental Toxicology and Ecology, unpublished

BASF (2000b): 2-(4-Diethylamino-2-hydroxybenzoyl)benzoesaeurehexylester: Acute toxicity study on the zebra fish (Brachydanio rerio HAM. and BUCH.) in a static system (96 hours). 17F0408/995068. Department of Experimental Toxicology and Ecology, unpublished

BASF (2000c): Physico-chemical properties of 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester. 99L00688. Department of central analytics, unpublished

BASF (2001a): 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesaeurehexylester - Determination of the inhibitory effect on the cell multiplication of unicellular green algae. 99/0765/60/1, date: 2001-04-11. Department of Experimental Toxicology and Ecology, unpublished

BASF (2001b): 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesaeurehexylester: Determination of the biodegradability in the manometric respirometry test. 99/0765/26/1. Department of Experimental Toxicology and Ecology. BASF

BASF (2005): Uvinul A Plus: Screening test on bioaccumulation in fish. 31F0496/045053. Department of Experimental Toxicology and Ecology. BASF

BASF (2006): Uvinul A Plus - Bioconcentration study in the Zebrafish (Danio rerio). 31F0496/045061. Department of Experimental Toxicology and Ecology. BASF, unpublished

BASF (2007): Influence of Uvinul A Plus to Daphnia magna in a reproduction test. 51E0636/029036, date: 2007-06-26. Institute for Biological Analytic und Consulting IBACON GmbH, Rossdorf, Germany, unpublished

BASF (2009): Uvinul A Plus: Daphnia magna Reproduction Test. 52E0636/023040, date: 2009-11-04. Experimental Toxicology and Ecology, unpublished

BASF (2010): Adsorption Coefficient (Koc) of "Uvinul A Plus Granular". 10L00183. GKA Competence Center Analytics. BASF

BASF (2013): Early Life-Stage Toxicity Test on the Fathead minnow (Pimephales promelas) in a flow through system. 50F0636/02E005, date: 2013-01-09. Experimental Toxicology and Ecology, unpublished

ECHA (2017): Registration Dossier hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate. European Chemicals Agency,. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/4877 (last accessed 25.04.2017)

Additional references

BASF 2018 BASF Comments on the CHL report by Federal Institute of Occupational Safety and Health (BAuA) on hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate, EC Number: 443-860-6, CAS Number: 302776-68-7, Index Number: 607-693-00-4.

BASF 2018a, Diethylamino Hydroxybenzoyl Hexyl Benzoate using *Daphnia magna* clone M10 (ECT Ökotoxikologie): BASF SE (2018), Report *Daphnia magna* reproduction test, unpublished data, report no. 51E0636/02E020.

BASF 2018b, Diethylamino Hydroxybenzoyl Hexyl Benzoate using *Daphnia magna* clone 5 (IBACON): BASF SE (2018) Report *Daphnia magna* reproduction test, unpublished data,report no. 51E0636/02E019.

BASF 2018c: Report Effects of the absence of soluble iron, Fe(II) in standard M4-Medium on the reproduction of the water flea *Daphnia magna* STRAUS 1820 (Screening Test), unpublished data, report no. 51E0000/18E006.

BASF SE (2009), Report Bestimmung der Wasserlöslichkeit in M4 Medium und in Milli-Q-Water, unpublished data, report no. 08E03159.

Biesinger K and Christensen G., 1972. Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna. Journal of the Fisheries Research Board of Canada, 29:1691-1700.

Bosnir, J., Puntarić, D., Cvetković, Z., Pollak, L., Barusić, L., Klarić, I., Miskulin, M., Puntarić, I., Puntarić, E., Milosević, M., 2013. Effects of magnesium, chromium, iron and zinc from food supplements on selected aquatic organisms. Collegium Antropologicum 37:965-971.

Dave, G., 1984. Effects on waterborne iron on growth, reproduction, survival and haemoglobin in Daphnia magna. Comparative. Biochemistry and Physiology, 78C: 433-438.

Hudsona, S.L., Dokeb, D.A., Gohlkea, J.M, 2016. The effect of a low iron diet and early life methylmercury exposure in Daphnia pule. Food Chem Toxicol, 89: 112–119.

Diethylamino Hydroxybenzoyl Hexyl Benzoate, CAS 302776-68-7: Declaration on additional data, June 2018.