Committee for Risk Assessment

RAC

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

2-(dimethylamino)-2-[(4-methylphenyl)methyl] -1-[4-(morpholin-4-yl)phenyl]butan-1-one

EC Number: 438-340-0
CAS Number: 119344-86-4

CLH-O-0000007134-80-01/F

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

Adopted

2 June2022

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-(DIMETHYLAMINO)-2-[(4-METHYLPHENYL)METHYL]-1-[4-(MORPHOLIN-4-YL)PHENYL]BUTAN-1-ONE

CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one

EC Number: **438-340-0**

CAS Number: **119344-86-4**

Index Number: **-**

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on behalf of the Austrian Competent Authority (Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology. Radetzkystraße 2, 1030 Vienna, Austria)

Version number: 02 **Date: June 2021**

Note: We would like to thank the CA of Slovakia for reviewing the dossier and the helpful comments.

CONTENTS

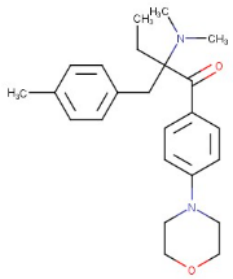
ABBREVIATIONS

AT	Austria
BCF	bioconcentration factor
bw	body weight
CAS	Chemcial Abstract Aervice
CCH	Compliance Check
CDF	cumulative distribution function
DDT	Dichlorodiphenyltrichloroethane
DMF	dimethylformamide
Drg	Danger
d	day
ECHA	European Chemcial Agency
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
Kow	Partition coefficient octanol/water
LOQ	limit of quantification
m/f	male/female
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
Pa	Pascal
QSAR	Quantitative Structure-Activity Relationship
UV	ultraviolet
UVCB	Chemical substances of unknown or variable composition

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)	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one
Other names (usual name, trade name, abbreviation)	Omnirad 379 JRCure 379 Keycure 8179 CGI 113 1-Butanone, 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(4-morpholinyl)phenyl]-
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	438-340-0
EC name (if available and appropriate)	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one
CAS number (if available)	119344-86-4
Other identity code (if available)	-
Molecular formula	C24 H32 N2 O2
Structural formula	 **(source:** European Chemicals Agency, http://echa.europa.eu/**)**
SMILES notation (if available)	CCC(CC1=CC=C(C)C=C1)(N(C)C)C(=O)C1=CC=C(C=C1)N1CCOCC1
Molecular weight or molecular weight range	380.5
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Racemate, ratio unknown
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

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)**
(R)-2-(4-methyl-benzyl)-2-(dimethylamino)-1-[4-(morpholin-4-yl)phenyl]butan-1-one	-	-	The racemat is self-classified as: Repr. 2, H361 Aquatic Chronic 4, H413 Aquatic Chronic 1, H410
(S)-2-(4-methyl-benzyl)-2-(dimethylamino)-1-[4-(morpholin-4-yl)phenyl]butan-1-one	-	-	

The substance is a multi-constituent substance (racemate).

Impurities are not relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one	438-340-0	119344-86-4	Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H360FD H400 H410	GHS08 GHS09 Dgr	H360FD H410	-	M=1 M=1	-

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

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*hazard class not assessed in this dossier*	No
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	*hazard class not assessed in this dossier*	No
Germ cell mutagenicity	*hazard class not assessed in this dossier*	No
Carcinogenicity	*data lacking*	No
Reproductive toxicity	Repr. 1B, H360FD	Yes
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*data conclusive but not sufficient for classification*	Yes
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	Aquatic Acute 1, H400, Aquatic Chronic 1, H410	Yes
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No previous discussions on classification and labelling documented.

The substance has 114 C&L notifications with self-classifications as Repr. 2, H361, Aquatic Chronic 4, H413 or Aquatic Chronic 1, H410 [ECHA dissemination site accessed December 2020].

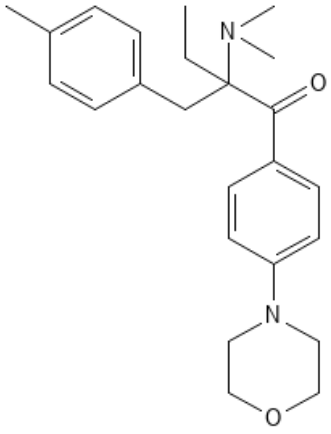
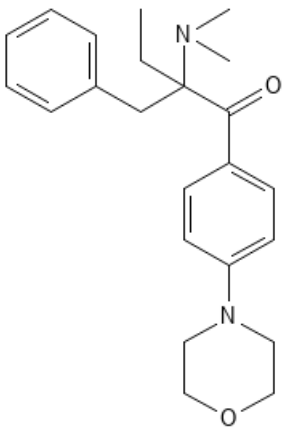
RAC general comment

2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one is a photo initiator used in formulation of UV inks for digital printing and preparations containing the photo initiator. There is wide dispersive indoor use by professional workers in UV inks and professional application of coatings and inks.

Read across

2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (EC 438-340-0), also known under the trade name Omnirad 379, is a photo initiator from the group of alpha amino ketones. A closely structurally related photo initiator Omnirad 369 (EC 404-360-3) was evaluated by RAC in 2016 and a harmonised classification as Repr. 1B; H360D was agreed mainly based on an increase in stillborn pups and postnatal mortality in a one-generation study. No classification was agreed for fertility. The dossier submitter (DS) for Omnirad 379 proposed read across from Omnirad 369 to Omnirad 379 for developmental toxicity but not for fertility. The structures of both substances are shown below; the only difference is that Omnirad 379 has an extra methyl group on one of the aromatic rings.

Structures of Omnirad 379 and Omnirad 369

	
Omnirad 379	Omnirad 369
CAS no. 119344-86-4	CAS no. 119313-12-1
EC no. 438-340-0	EC no. 404-360-3
2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one	2-benzyl-2-dimethylamino-4′-morpholinobutyrophenone
Read across: target substance	Read across: source substance

RAC notes that the substances have very similar structures and similar toxicological profiles: low acute toxicity, do not cause significant local effects, non-genotoxic, target organs are liver and kidney, and both cause increased postnatal mortality. However, only Omnirad 379 was found to cause testicular degeneration in the available studies.

Given the close structural similarity and also toxicological similarity between the two substances for most endpoints, RAC agrees to use the studies with Omnirad 369 as supporting information in the classification of Omnirad 379. The read across should be applied consistently for both fertility and development. As to STOT RE, relevant effects of both substances are broadly similar and consideration of the repeated dose studies with Omnirad 369 has no significant impact on the assessment.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

Further detail on need of action at Community level

In the course of a CCH further data has been generated. Due to differences in self-classification a harmonized classification for environment is proposed in this dossier.

5 IDENTIFIED USES

Table 5: The following uses are indicated at ECHA dissemination site [accessed December, 2020]:

	Use(s)	Technical function
Manufacture	-	-
Formulation	Formulation of UV inks for digital printing Industrial manufacture of coatings and inks Formulation of preparations containing the photoinitiator	Photoinitiator
Uses at industrial sites	Industrial use of the photoinitiator in UV-inks Industrial application of coatings and inks Industrial use of UV inks for digital printing	Photoinitiator
Uses by professional workers	Wide dispersive indoor use of photoinitiator in UV-inks Professional application of coatings and inks Widespread use by professional workers: UV inks for digital printing	Photoinitiator

Consumer Uses	-	-
Article service life	Use of printed or coated article (plastic and paper) Photoinitiator in UV-inks	Photoinitiator

6 DATA SOURCES

ECHA dissemination site: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15616/1/2 and https://echa.europa.eu/de/registration-dossier/-/registered-dossier/7943/1/1

Original study reports for EC 438-340-0 have been provided by registrants and used for evaluation.

Read across to the similar substance 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone (EC 404-360-3) has been applied for the endpoints toxicity to reproduction and repeated dose toxicity. For further information see Annex I.

7 PHYSICOCHEMICAL PROPERTIES

For this substance two registrations are available at ECHA dissemination site (see Chapter 6). When diverging values for relevant physicochemical properties are reported both are presented in the table, indicated as (1) and (2); relevant properties for this dossier are discussed below.

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid powder, light yellow	ECHA dissemination site [Feb, 2020]	-
Melting/freezing point	(1) 68°C (2) >92.8 ≤ 95.5 °C	ECHA dissemination site [Feb, 2020]	(1) OECD 102 (2) OECD 102
Boiling point	decomposition at 270°C	ECHA dissemination site [Feb, 2020]	OECD 103
Relative density	(1) 1.16 (2) 1.17	ECHA dissemination site [Feb, 2020]	(1) OECD 109 (2) OECD 109
Vapour pressure	(1) 0 Pa (25°C) (2) 40 Pa (20°C)	ECHA dissemination site [Feb, 2020]	(1) Calculated (2) OECD 104
Surface tension	70.4 mN/m (20°C)	ECHA dissemination site [Feb, 2020]	OECD 115
Water solubility	1.9 mg/L (20°C); pH 6.8	ECHA dissemination site [Feb, 2002]	OECD 105, not valid (measured results differ by 32% , due to instability of test item)

Property	Value	Reference	Comment (e.g. measured or estimated)
	2.8 mg/L± 0.2 mg/L (20°C), column elution method; pH 6.1 3.3 mg/L ± 0.5 mg/L, (20°C) (flask method), pH ≥5.9 - ≤6.3	Anonymous, 2013c	OECD 105, valid
Partition coefficient n-octanol/water	(1) Log Pow = 4.1 (25°C) (2) Log Pow = 5.73 (25°C) (2) Log Pow = 4	ECHA dissemination site [Feb, 2020]	(1) OECD 117 (2) OECD 117 (2) QSAR KOWWIN v1.68 EPIsuit 4.0
Flash point	178°C (at 1013 hPa)	ECHA dissemination site [Feb, 2020]	ISO 2719
Flammability	Not highly flammable	ECHA dissemination site [Feb, 2020]	EU Method A.10
Explosive properties	No explosive properties	ECHA dissemination site [Feb, 2020]	Data waiving
Self-ignition temperature	-	ECHA dissemination site [Feb, 2020]	Waiving: melting point < 160°C
Oxidising properties	-	ECHA dissemination site [Feb, 2020]	Waiving: no oxidising prop.
Granulometry	MMD= 16.7µm D10= 1.5µm D90= 40µm range: ~ 0.3 - 100µm	ECHA dissemination site [Feb, 2020]	EC Guidance Document, ECB/TM/February 1996.
Stability in organic solvents and identity of relevant degradation products	-	ECHA dissemination site [Feb, 2020]	Waiving: stability not considered critical
Dissociation constant	pKa 6.22 (20°C)	ECHA dissemination site [Feb, 2020]	(Q)SAR modelling
Viscosity	-	ECHA dissemination site [Feb, 2020]	Waiving: solid

QSAR-model MPBPVP v1.43 (EPISUITE 4.1) predicts a vapour pressure of 3.29 x 10^{-7}Pa at 25°C. In this context the predicted vapour pressure is equal to 0 Pa at 25°C; it is considered to be conclusive and acceptable indicating, that the vapour pressure is low. On the other hand, the measured value of 40 Pa cannot be excluded on a technical level, assuming that the higher vapour pressure is determined by lower volatile impurities, constituents or solvent residues. The main constituent is considered to be non-volatile.

As the original study of the log Pow measurement with a Log Pow = 5.73 (25°C) was not accessible for review, the deviation of this value from the others could not be evaluated. As the measured value of 4.1 and the estimated one of 4 are in the same range, they are considered to be more reliable. In addition, the measurement performed did not reveal any obvious errors in performance.

For water solubility three different values are reported by registrants. Referring to the measured water solubility of 1.9 mg/L, partial photolytical degradation of the parent compound was indicated in the study,

although it was attempted to avoid photolysis. This might explain the lower value measured in comparison to the other ones. Photolytical degradation was also tried to be excluded for the tests leading to the results: 2.8 mg/L± 0.2 mg/L (20°C) (column elution method) and 3.3 mg/L ± 0.5 mg/L (20°C) (flask method), reported in one study. Indications for photolytical degradation were not identified. The results are in agreement to each other and in the same range. Regarding the given values for water solubility and the higher certainty of the column elution, 2.8 mg/L ± 0.2 mg/L (20°C) is considered to be the most valid one among these values and is taken into account for the following assessment.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No toxicokinetic study with EC 438-340-0 is available. However, based on the physicochemical properties a rought estimation on absorption has been done by registrants.

The substance has a molecular weight of 380.5 g/mol and is solid at room temperature. It is not susceptible to acid-catalysed hydrolysis as shown in the hydrolysis study (OECD 111) at pH 4 (see Chapter 11).

Solubility in water is generally low, but highest at acidic pH. The log Pow of 4.1 at neutral pH indicates a low potential for bio-accumulation. The dissolution in water and distribution in octanol/water are pH-dependant since the molecule has a tertiary amine function that can protonated leading to an ionic structure revealing a higher affinity for the aqueous phase.

The relatively low water and high fat solubility as well as the moderate molecular weight suggest reasonably good uptake of the substance via all routes of exposure. This is supported by systemic effects seen after repeated oral exposure (14-day study, 28-day study, Reproduction/Developmental Toxicity Screening Test).

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not evaluated.

10.2 Acute toxicity - dermal route

Not evaluated.

10.3 Acute toxicity - inhalation route

No data available.

10.4 Skin corrosion/irritation

Not evaluated.

10.5 Not evaluated.Serious eye damage/eye irritation

Not evaluated.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

Not evaluated.

10.8 Germ cell mutagenicity

Not evaluated.

10.9 Carcinogenicity

No data available.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

For evaluation of effects of 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one on sexual function and fertility one Reproduction/Developmental Toxicity Screening study and one 28-day study are available as well as a One-Generation Reproduction Toxicity Study with the read-across substance 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone. For read-across justification see Annex I.

Table 7: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 421 Reproduction / Developmental Toxicity Screening Test GLP Rat, Crl:WI(Han) N=10/sex/group	Test substance: 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one 0, 20, 60, 200 mg/kg bw 7d/week 28 days (males) 42 - 52 days (females) Oral, gavage Vehicle: polyethylene glycol	NOAEL (systemic, m) = 20 mg/kg bw NOAEL (fertility, m) = 60 mg/kg bw <u>200 mg/kg bw:</u> Reduced bw gain in males (m); bw loss in females (f) in week 1 of treatment, decreased food consumption in f Decreased terminal body weights in m, reduced epididymides weight (-17%); intraluminal cell debris of the epididymides in 8/10 males (up to moderate degree), oligospermia of the epididymides in 4/10 males (up to slight degree) Germ cell exfoliating into the lumen of seminiferous tubules of the testes (without degeneration) in 9/10 males (up to moderate degree) Lymphoid atrophy (moderate) of the thymus in 2/10 females <u>60 mg/kg bw:</u> Decreased terminal body weights in m, reduced bw gain in m, slightly higher testis weights	Anonymous (2013b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD 407 28-day oral toxicity study GLP Rat, HanBrl:WIST (SPF) 0, 450 mg/kg bw/day: N=10 m + 10 f 15, 50, 150 mg/kg bw/day: N=5 m + 5 f	Test substance: 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one 0, 15, 50, 150 and 450 mg/kg bw 28 days, 7 days/week Oral, gavage Vehicle: polyethylene glycol	NOAEL (systemic tox) = 50 mg/kg bw NOAEL (fertility, m) = 150 mg/kg bw <u>450 mg/kg bw:</u> reduced weights of testes (-49%, p<0.01) and epididymides, (-34%, p<0.01) small testes in 5/5 m at the end of treatment and in 4/5 males at the end of recovery moderate to markedly reduced spermatogenesis in 5/5 (not reversible) occurrence of spermatic giant cells in 3/5, marked tubular atrophy in 1/5 (not reversible) epididymides containing cellular debris in 5/5 (slight to marked) and severely reduced amount of spermatozoa (not reversible) No fertility relevant findings in lower dose groups	Anonymous (2002a)
OECD 415 One-Generation Reproduction Toxicity Study GLP Rat (Wistar) N=20/sex/group	Test substance: 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone (read-across) 0, 30, 100, 300 mg/kg bw males: 110 d = ca. 16 weeks females: 126 d = 18 weeks Oral, gavage Vehicle: polyethylene glycol	NOAEL (fertility, m) = 100 mg/kg bw NOAEL (systemic tox) = 100 mg/kg bw Changes in weight of male reproductive organs at 300 mg/kg bw (testes ↑, prostate ↓, seminal vesicle ↓) No histopathological/ functional changes No effects in female reproductive organs	Anonymous (2011) [cited in BASF, 2015]

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

In a **14-day dose range finding study** dose levels of 150 and 300 mg/kg bw/day were tested. Study details see Chapter 10.12. As a consequence dose levels for the main study were 20, 60, and 200 mg/kg bw/day.

In the **main OECD 421 study** (Anonymous, 2013b) Crl:WI(Han) rats were exposed (oral, gavage) to 0, 20, 60 or 200 mg/kg bw/day. Males were exposed for 28 days (two weeks prior to mating, mating, up to termination), females for 42-52 days (two weeks prior to mating, mating, during post-coitum, at least 3 days of lactation). Further study details are described in Chapter 10.12 (specific target organ toxicity - repeated exposure).

No mortality occurred during the study period in parental animals. At 200 mg/kg bw/day, two females (#71 and 79) were euthanized on day 1 or 2 of lactation as they showed a total litter loss. General clinical signs (1 to 2 hours after dosing) at 200 mg/kg bw were piloerection (6f), hunched posture (3f) observed on some days during premating and in week 3 and 4 after mating. One high dosed female (#71) also showed lethargy and

pale appearance on a single day. Salivation was seen after dosing at 60 and 200 mg/kg bw/day in week four after mating. At the highest dose reduced body weight gain was noted for males; females showed bw loss during the first week of treatment (but recovering during the remainder of the study) which was accompanied by a decreased food consumption. At 200 mg/kg bw during lactation also decreased food consumption was reported. In males food consumption was not affected (for details see Chapter 10.12)

Macroscopic examination of males showed nodule at the epididymides and enlarged mandibular lymph nodes in some animals without dose response. In females alopecia was seen in singular animals and focus/foci on the clitoral glands were seen in one control female (1/10) and two high dose females (2/8). These findings were reported to be within the background range of findings that are encountered among rats of this age and strain. For the two females that showed a total litter loss at 200 mg/kg bw (necropsy was within 24h) macroscopic findings in kidneys and/or thymus are reported. Female #71 showed general pale discolouration, many reddish foci on the kidneys and gelatinous thymus. Female #79 showed the thymus reduced in size.

Only organ weights of testes and epididymides were examined (Table 8). For males terminal body weights were decreased at 60 and 200 mg/kg bw/day. Absolute and relative weight of the epididymides was statistically significantly reduced at 200 mg/kg bw/day (-17%). A slightly higher mean testes weight in the mid dose group resulted in a statistically significant higher organ/body weight ratio (control 0.93±0.05 versus 60 mg/kg bw 1.01*±0.06). No dose response for this effect was observed.

Table 8: Organ weights [gram], males (mean ± St.Dev, n=number of animals) (Anonymous, 2013b).

Parameter	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
males				
Body weight at end of treatment	390 ± 16 n=10	383 ± 18 n=10	371* ± 9 n=10	362** ± 14 n=10
Testes	3.64 ± 0.21 n=10	3.49 ± 0.25 n=10	3.74 ± 0.22 n=10	3.45 ± 0.19 n=10
Epididymides	1.206 ± 0.119 n=10	1.130 ± 0.102 n=10	1.158 ± 0.098 n=10	0.998** ± 0.124 n=10

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Males treated with 200 mg/kg bw showed the following microscopic findings in testes and epididymides: intraluminal cell debris of the epididymides in 8/10 males (up to moderate degree), oligospermia of the epididymides in 4/10 males (up to slight degree), germ cell exfoliating into the lumen of seminiferous tubules of the testes (without degeneration) in 9/10 males (up to moderate degree). However, no impaired fertility has been seen (Table 9); but this may be due to the higher sperm reserve in rats versus humans (Mangelsdorf & Buschmann, 2003).

For females treated with 200 mg/kg bw findings in the kidneys and thymus were recorded: glomerular and tubular necrosis (marked) of the kidneys in 1/10 females (noted at necropsy as many reddish foci, both sides), lymphoid atrophy (moderate) of the thymus in 2/10 females (#71 and 79), noted at necropsy as gelatinous or reduced size.

Most females mated within 4 days of pairing; one female (#46) of the control group did not mate within the 14-day pairing period. One control female (#45) and one female at 60 mg/kg bw/day (#64) were not pregnant. No cause for the failure to sire or deliver healthy offspring could be established from the sections examined.

Mating, fertility and conception indices, precoital time, and number of corpora lutea and implantation sites were unaffected by treatment. Spermatogenic staging profiles were normal for all males. No toxicologically relevant effects on reproductive parameters were noted up to 200 mg/kg bw/day (see Table 9 and Table 10).

Table 9: Reproduction data (Anonymous, 2013b).

Parameter	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
Females paired	10	10	10	10
Females mated	9	10	10	10
Pregnant females	8	10	9	10
F with living pups on day 1	8	10	9	9
Mating index (%) $(f_{mated}/f_{paired})x100$	90	100	100	100
Fertility index (%) $(f_{pregnant}/f_{paired})x100$	80	100	90	100
Conception index (%) $(f_{pregnant}/f_{mated})x100$	89	100	90	100
Gestation index (5) $(f_{living\ pups\ on\ day\ 1}/f_{pregnant})x100$	100	100	100	90

Table 10: Female fertility parameters (mean ± St.Dev, n=number of animals) (Anonymous, 2013b).

Parameter	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
Corpora lutea	13.5 ± 2.4 n=8	12.8 ± 1.3 n=10	13.4 ± 2.1 n=9	13.2 ± 2.3 n=10
Implantations	12.5 ± 1.7 n=8	11.2 ± 2.0 n=10	12.2 ± 1.6 n=9	12.6 ± 2.5 n=10

Developmental parameters examined in the screening study are presented in Chapter 10.10.5.

In a **28-day study** HanBrl:WIST (SPF) rats were orally exposed (gavage) to concentrations of 0, 15, 50, 150 or 450 mg/kg bw/d of the test substance (Anonymous, 2002a). 10 males and 10 females were used for the control and the high dose group (including 14 day recovery group), 5 males and 5 females for the remaining groups. The study is in detail described in Chapter 10.12 and only findings relevant for fertility are reported here.

No mortality was observed until scheduled necropsy. Clinical signs, consisting of piloerection, hunched posture, emaciation and waggling gait (days 9-12), were observed in males and females at 450 mg/kg/day only on some days and were reversible. The mean body weights were reduced in both sexes treated with 450 mg/kg/day from treatment day 8 onwards when compared with the control rats. On day 28 a reduction of -11.3% and -13.8% in males and females, respectively, is documented. During the recovery period, the mean body weights of rats in this dose group remained lower than those of the controls, although the mean body weight gain improved. In males treated with 150 mg/kg/day a reduction was seen from day 22 onwards with a reduction of -7.8% on treatment day 28. The mean body weights of females treated with 150 mg/kg/day were unaffected. The absolute mean daily food consumption was reduced during the first measurement interval (days 1-8) in males and females at 450 mg/kg/day when compared with controls, but improved thereafter to

levels comparable to that of the controls. This trend was also seen in the relative food consumption. The mean daily food consumption of the remaining groups was unaffected.

Hematology and clinical biochemistry showed substance related changes at 150 and 450 mg/kg bw (see Chapter 10.12 for further details).

Examination of male organ weights after four weeks of treatment with 450 mg/kg/day showed test item related changes in absolute organ weights and organ-to-brain weight ratios in the livers (+15% $p<0.05$,), kidneys (+13%, $p<0.01$), testes (-49%, $p<0.01$) and epididymides (-34%, $p<0.01$) of rats (Table 11).

In females, test item-related changes in absolute organ weights were noted as increased liver weights (+42%, $p<0.01$). A similar change was noted in the liver-to-brain weight ratio. Increased absolute ovary weights (+23%, not significant) and ovary-to-body weight ratios (+41%, $p<0.05$), noted in females at 450 mg/kg/day, were considered likely to represent normal variations due to estrus, as there were no microscopical alterations which correlated to this finding.

Macroscopic observations show small testes ("diminished in size") in 5/5 males at 450 mg/kg/day at the end of treatment and in 4/5 males at the end of the two week recovery period. No testes findings in lower dose groups. The remaining macroscopic findings were considered to be typical background findings unrelated to the treatment with the test item.

Table 11: Organ weights [gram] for males and females after 28 days of treatment and recovery (mean ± St.Dev, n=5 for all parameters) (Anonymous, 2002a).

Parameter [unit]	Control group	15 mg/kg bw/d	50 mg/kg bw/d	150 mg/kg bw/d	450 mg/kg bw/d	Control recovery	450 mg/kg bw/d recovery
males							
Body weight	296.802 ± 22.420	296.018 ± 9.262	296.316 ± 11.155	278.752 ±15.524	264.908** ± 11.481	349.948 ± 27.066	312.664** ± 17.685
Testes	3.66 ± 0.39	3.32 ± 0.17	3.33 ± 0.29	3.49 ± 0.35	1.87** ± 0.42	3.73 ± 0.19	2.14**±0.97
Epididymides	1.062 ± 0.116	1.051 ± 0.122	1.001 ± 0.034	0.959 ± 0.033	0.704** ± 0.102	1.260 ± 0.099	0.717** ± 0.153
females							
Body weight	185.054 ± 12.866	173.258 ± 8.302	182.502 ± 17.054	174.978 ± 8.459	162.966* ± 13.367	192.306 ± 12.776	172.984* ± 9.557
Ovary	0.101 ± 0.022	0.119 ± 0.027	0.095 ± 0.020	0.098 ± 0.019	0.124 ± 0.023	0.113 ± 0.015	0.130 ± 0.023

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

Microscopic examination demonstrated that testes of all 5 males at 450 mg/kg/day exhibited moderately to markedly reduced spermatogenesis, 3/5 males had slight to marked occurrence of spermatic giant cells, and one male had marked tubular atrophy. At the end of recovery period, testes in four of five males at 450 mg/kg/day still were affected and exhibited markedly reduced spermatogenesis and slight to marked tubular atrophy. In one of these individuals, minimal occurrence of spermatic giant cells was observed. At the end of treatment high dosed males showed epididymides containing cellular debris (slight ot marked) and severely reduced amount of spermatozoa. These changes were considered to be secondary to the effect on testes, coincided in three males, whereas in one additional male only cellular debris and in another male only reduced spermatozoa were observed. Following the recovery period, minimal to slight cellular debris occurred in all five males and severely reduced spermatozoa were recorded in four of five individuals at 450 mg/kg/day. The findings in the male genital system correlated with macroscopic observation of small testes, as well as with reductions in organ weight of testes and epididymides.
Effects on other organs and general parental toxicity are described in Chapter 10.12.

In a **one-generation-reproductive toxicity study** the source substance EC 404-360-3 was administered daily (gavage) to Wistar rats at doses of 0, 30, 100 or 300 mg/kg bw. Health state was checked daily, food consumption and body weights weekly. At necropsy, all pups were examined macroscopically (including weight determinations of brain, spleen and thymus in one pup/sex/litter). All F0 parental animals were assessed by gross pathology (including weight determinations of several organs) and affected organs were subjected to histopathological examination, special attention being paid to the organs of the reproductive system (Anonymous, 2011; BASF, 2015).

Reproductive indices were not affected, and in females no treatment-related effects were found on reproductive organs. In males in the high dose group, an absolute and relative testes weight increase was observed (107% and 113%, respectively), as well as an absolute and relative prostate weight decrease (80% and 85%, respectively) and an absolute, but not relative, seminal vesicles weight decrease (87%). However, no histopathological findings were observed in reproductive organs of male and female rats at 300 mg/kg bw (RAC opinion, 2016).

Table 12: Absolute organ weight changes of male reproduction organs in % (Anonymous, 2011).

	0 mg/kg bw	30 mg/kg bw	100 mg/kg bw	300 mg/kg bw
Testes	100	98	100	107*
Prostate	100	97	100	80**
Cauda epididymis	100	100	100	100
Epididymides	100	97	100	100
Seminal vesicle	100	97	99	87**

*p <= 0.05;**p <= 0.01, compared to control group

In the CLH report (BASF, 2015) for the source substance (EC 404-360-3) also three subacute oral toxicity studies in rats are described with special investigations in relation to fertility: In a 14-day range-finding study at dose levels ranging from 100-3000 mg/kg bw/day, the gonad weights were not affected (Anonymous, 1989a). In the subsequent main study of 28-day duration, dose levels ranging from 10-500 mg/kg bw/day did not reveal macroscopic findings on reproductive organs, and therefore their weights were not determined and histopathology was not performed (Anonymous, 1989b). In a 28-day range-finding study to the one-generation study, with dose levels of 100 and 500/250 mg/kg bw/day (500 mg/kg bw were not tolerated by the rats and reduced to 250 mg/kg bw after 9 days), no treatment-related effects were observed on histopathology of testes and epididymides, sperm motility and spermatogenesis (Anonymous, 2009) (RAC, 2016). For more details see Annex I, Table 53.

10.10.3 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when

there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

For evaluation of this endpoint a screening study and a 28-day study with EC 438-340-0 itself are available showing effects on the male reproductive system.

In the OECD 421 study males showed decreased terminal body weights at 60 and 200 mg/kg bw (up to -7%). Absolute and relative weight of the epididymides was statistically significantly reduced at 200 mg/kg bw/day (-17%). A slightly higher mean testes weight in the mid dose group was observed but no dose response for this effect. Macroscopic examination of males showed nodule at the epididymides. At 200 mg/kg microscopic findings in testes and epididymides at 200 mg/kg bw were intraluminal cell debris of the epididymides in 8/10 males (up to moderate degree), oligospermia of the epididymides in 4/10 males (up to slight degree), germ cell exfoliating into the lumen of seminiferous tubules of the testes (without degeneration) in 9/10 males (up to moderate degree). Effects on fertility have not been seen maybe due to the high sperm reserve in rats (Mangelsdorf & Buschmann, 2003). In addition it has to be noted that the statistic power of a screening study is low.

In an 28-day study exposure to 450 mg/kg/day showed effects on testes (-49%, $p<0.01$) and epididymides (-34%, $p<0.01$) weights. Small testes has been described even after recovery. Microscopic examination demonstrated that testes of high dosed males exhibited moderately to markedly reduced spermatogenesis, slight to marked occurrence of spermatic giant cells, and marked tubular atrophy (in one male). Effects were still present after recovery. Effects in the epididymides were considered to be secondary to the effect on testes. General toxicity in males was described at 450 mg/kg bw with reduced mean bw (-11,3%), effects on haematology (decreased hemoglobin and hematocrit values), increased liver (+15%) and kidneys (+13%, hyaline changes) weight and in a minor extend at 150 mg/kg bw.

For the source substance EC 404-360-3 a One-Generation Reproduction Toxicity Study is available showing changes in weight of male reproductive organs at 300 mg/kg bw (testes ↑, prostate ↓, seminal vesicle ↓) but no histopathological/ functional changes. In three subacute oral toxicity studies (with special investigations in relation to fertility) in rats with the source substance no effects were seen.

10.10.4 Adverse effects on development

Table 13: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD 421 Reproduction / Developmental Toxicity Screening Test GLP	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one 0, 20, 60, 200 mg/kg bw	NOAEL (maternal tox) = 60 mg/kg bw NOAEL (development) = 20 mg/kg bw <u>200 mg/kg bw:</u> bw loss in f week 1 of treatment, decreased food consumption in f; lymphoid atrophy (moderate) of the	Anonymous (2013b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Rat, Crl:WI(Han) N=10/sex/group	28 days (males) 42 - 52 days (females) Oral, gavage Vehicle: polyethylene glycol	thymus in 2/10 females Signif. reduced viability index (87.9%) Dead pups (20) at first litter check from 2/10 females Postnatal loss (-12.1%) in 4/10 litters Reduced pup body weights (-20% on day 4) <u>60 mg/kg bw:</u> in total 5 dead pups in 2 litters: - first litter check: four dead pups in 1/10 females - postnatal loss: one pup in 1/10 litters	
OECD 415 One-Generation Reproduction Toxicity Study GLP Rat (Wistar) N=20/sex/group	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone (read-across) 0, 30, 100, 300 mg/kg bw males: 110 d = ca. 16 weeks females: 126 d = 18 weeks Oral, gavage Vehicle: polyethylene glycol	NOAEL (systemic tox) = 100 mg/kg bw NOAEL (development) = 30 mg/kg bw Relative liver weight increase (+50% f; +34% m) with central/midzonal hypertrophy at 300 mg/kg bw Reduced food consumption and body weight gain during gestation and lactation in females at 300 mg/kg bw <u>300 mg/kg bw:</u> Signif. reduced viability index (86%) Stillborn pups (9) in 8/17 females (signif) Postnatal mortality (days 0-4): 32% (signif) Decrease in pup weight (e.g -16% on day 4) (signif) <u>100 mg/kg bw:</u> Stillborn pups (6) in 5/17 females (signif) Postnatal mortality (days 0-4): 7%	Anonymous (2011)

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

The **Reproduction/Developmental Toxicity Screening Test** is described in detail in Chapter 10.12.1. Crl:WI(Han) rats were exposed (oral, gavage) to 0, 20, 60 or 200 mg /kg bw/day. Males were exposed for 28 days (two weeks prior to mating, mating, up to termination), females for 42-52 days (two weeks prior to mating, mating, during post-coitum, at least 3 days of lactation). For reporting of general signs of toxicity on parental animals see Chapter 10.12.

At 200 mg/kg bw/day developmental toxicity was noted consisting of an increased number of dead pups at first litter check and postnatal loss, and a decreased number of living pups at first litter check as well as decreased viability index and body weights of the pups (see Table 14 and Table 15). All dead pups at first litter

check (n=20) were from litters #71 and #79. In addition, seven out of twelve postnatal losses were from litter #79; the remaining five postnatal losses were from litters #73, 76 and 78.

In total (dead at first check and postnatal loss) one pup of the control group, two pups at 20 mg/kg bw/day, five pups at 60 mg/kg bw/day and thirtytwo pups at 200 mg/kg bw/day were found dead (at first litter check) or missing (cannibalized) during the first days of lactation. Pups that were found dead at 200 mg/kg bw/day showed absence of milk in the stomach, cannibalism, cold appearance, and beginning autolysis. One pup at this dose showed a blue spot on the snout. At the other dose groups dead pups also showed absence of milk in the stomach. In addition they showed scabs at the abdomen or snout, blue spot on the snout, pale appearance, wound or scabs at the cervical region and they were affected by beginning autolysis. These findings were considered of no toxicological relevance by the study authors as the nature and incidence of these findings remained within the range considered normal for pups of this age. However, the absence of milk in the stomach of dead pups, despite the described normal maternal care, may indicate that the pups were not able to suckle (see also Chapter 10.10.7). This could be evidence for a direct effect of the test substance on the pups or related to maternal toxicity. However, there is no evidence of maternal toxicity in the mid dose and only slight maternal toxicity in top dose. In addition there is no evidence that the observed effects are secondary non-specific consequence of the observed maternal toxicity seen in the top dose, which would be required according to the CLP guidance.

Table 14: Developmental data and historical control data (Anonymous, 2013b).

Parameter	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d	Historical control data
Litters total	8	10	9	10	
Duration of gestation [mean±St.Dev.]	21.5 ± 0.5 n=8	21.3 ± 0.5 n=10	21.7 ± 0.7 n=9	21.4 ± 0.7 n=10	21.3 ± 0.52 n=907
Dead pups at first litter check					
- Litters affected (#)	0	2	1[c]	2[a]	-
- Total (#)	0	2	4	20	-
- Mean±St.Dev	0.0 ± 0.0 n=8	0.2 ± 0.4 n=10	0.4 ± 1.3 n=9	2.0 ± 4.4 n=10	0.1 ± 0.31 n=907
Living pups at first litter check					
- % of m/f	48/52	59/41	45/55	46/54	-
- Total (#)	92	106	98	99	-
- Mean±St.Dev	11.5 ± 2.0 n=8	10.6 ± 1.8 n=10	10.9 ± 2.5 n=9	9.9 ± 4.2 n=10	11.8 ± 2.52 n=907
Postnatal loss					
- % of living pups	1.1	0.0	1.0	12.1	-
- Litters affected (#)	1	0	1[d]	4[b]	-
- Total (#)	1	0	1	12[##]	-
- Mean±St.Dev	0.1 ± 0.4 n=8	0.0 ± 0.0 n=10	0.1 ± 0.3 n=9	1.2 ± 2.2 n=10	0.1 ± 0.36 n=907
Viability index (Number of alive pups before planned necropsy /	98.9	100.0	99.0	87.9[##]	99%

Number of pups born alive) *100					

/ ## Fisher's Exact test significant at 5% (#) or 1% (##) level
+/++ Steel-test significant at 5% (+) or 1% (++) level
[a] females # 71, 79
[b] females # 73, 76, 78, 79
[c] female # 63
[d] female # 66

No toxicologically relevant effects on gestation index and duration, parturition and maternal care were observed.

Pup weights were unaffected in the 20 and 60 mg/kg bw/day dose group. At 200 mg/kg bw/day, statistically significantly reduced pup body weights were noted for both sexes on days 1 (-15%) and 4 (-20%) of lactation.

For parental toxicity see Chapter 10.12.1.

Table 15: Pups body weight in gram [mean+-St.Dev, n=number of litters] (Anonymous, 2013b).

Day	Sex	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
1	m	6.3 ± 0.6 n=8	6.2 ± 0.7 n=10	6.3 ± 0.7 n=9	5.4* ± 0.5 n=9
	f	6.0 ± 0.7 n=8	6.0 ± 0.6 n=10	6.0 ± 0.6 n=9	5.1* ± 0.6 n=9
	m+f	6.2 ± 0.6 n=8	6.1 ± 0.6 n=10	6.1 ± 0.7 n=9	5.3* ± 0.6 n=9
4	m	9.4 ± 1.0 n=8	9.2 ± 1.2 n=10	9.3 ± 1.3 n=9	7.5** ± 0.9 n=8
	f	9.0 ± 1.2 n=8	9.0 ± 1.2 n=10	9.0 ± 1.3 n=9	7.3* ± 1.1 n=8
	m+f	9.2 ± 1.1 n=8	9.1 ± 1.2 n=10	9.1 ± 1.3 n=9	7.4* ± 1.0 n=8

With the source substance EC 404-360-3 a **One-Generation-Reproduction-Toxicity study** (Anonymous, 2011) is available (read-across). In this GLP study Wistar rats (n=20/sex/group) were exposed to doses of 30, 100 and 300 mg/kg body weight/day (gavage). Study is described in more detail in Chapter 10.10.2 and below.

Females had a significantly increased number of stillborn pups at mid and high dose, increased postnatal mortality at 300 mg/kg bw and a decrease in pup body weight at the mid and high dose (see Table 16, Table 17 and Table 18). Parameters for the mid dose group were within the historical range but where considered as relevant by RAC due to the dose-response seen. Clinical and/or gross necropsy examinations of the F1 revealed no adverse findings. Effects seen were considered as severe, in particular the first two which, when combined, indicate a rather strong effect (approximately 20% mortality at the high dose). At the high dose also several maternal effects have been observed but RAC considered the developmental effects not to be secondary effects as first signs of reproductive toxicity were already seen at the mid dose (RAC opinion, 2016).

Table 16: Female delivery data with source substance EC 404-360-3 (read-across) (Anonymous, 2011).

Parameter	Control group	30 mg/kg bw/d	100 mg/kg bw/d	300 mg/kg bw/d
Litters total	18	19	17	17
Females with stillborn pups N (%)	0 (0)	1 (5.3)	5 (29)*	8 (47)*
Pups delivered per dam (mean)	10.8	10.1	11.2	9.5
Live birth index (%)	100	99	97	94
Stillborn pups N (%)	0 (0)	2 (1.0)	6 (3.2)*	9 (5.6)*
Viability index N (%)	194 (100)	185 (98)	181 (98)	131 (86)*
Lactation index N (%)	134 (100)	139 (100)	129 (99)	103 (99)

*p ≤0.05, compared to control group

Table 17: Postnatal mortality [n=number of pups) (Anonymous, 2011).

Day of observation	Control group	30 mg/kg bw/d	100 mg/kg bw/d	300 mg/kg bw/d
Day 0	0	1	0	4
Days 1-4	0	3	3	18
Days 5-7	0	0	1	0
Days 8-14	0	0	0	1
Days 15-21	0	0	0	0
Pups surviving days 0-4	194	185	181	131**
Pups surviving days 4-21	134	139	129	103

**p ≤0.05, compared to control group

Table 18: Pup weights in gram (group means) (Anonymous, 2011).

Day	Sex	Control group	30 mg/kg bw/d	100 mg/kg bw/d	300 mg/kg bw/d
1	m+f	6.3	6.3	5.9	5.5*
4	m+f	9.3	9.5	8.8	7.8*
7	m+f	14.7	15.0	14.0	11.1*
14	m+f	29.3	29.5	28.1	22.6*
21	m+f	46.6	46.3	44.3	36.9*

*p <= 0.05;** p <= 0.01, compared to control group

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Adverse effects on development

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

In an OECD 421 study EC 438-340-0 shows clear developmental toxicity at a concentration of 200 mg/kg bw. Dead pups at first litter check in two females, a significantly reduced viability index (87.9%), postnatal loss (-12.1%) and reduced pup weights (up to -20%) are documented. At 60 mg/kg bw slight effects (5 dead pups in 2 litters) are documented. Maternal toxicity is seen at the highest concentration with body weight loss in females in week 1 of treatment (recovering during the remainder of the study), decreased food consumption in females (on some points in time) and lymphoid atrophy (moderate) of the thymus in 2/10 females (showing total litter loss). Occasionally clinical signs were observed in some of the dams (6/10 piloerection, 3/10 hunched posture, 1/10 pale & lethargic on 1 day). Based on this study a NOAEL (maternal) of 60 mg/kg bw can be derived. In general the observed maternal toxicity in the top dose was not severe. The developmental toxicity seen is considered not to be a secondary non-specific consequence of maternal toxicity. A NOAEL (development) of 20 mg/kg bw can be derived.

In an OECD 415 study with the source substance EC 404-360-3 a significantly increased number of stillborn pups at 100 and 300 mg/kg bw, increased postnatal mortality at 300 mg/kg bw and a decrease in pup body weight at the mid and high dose was described. At the high dose also several maternal effects have been observed but RAC considered the developmental effects not to be secondary effects (RAC opinion, 2016). A NOAEL (development) of 30 mg/kg bw has been derived. Based on this study the substance has received a harmonized classification as Repr. 1B, H360D.

A rationale for read-across is given in Annex I of this report.

10.10.7 Adverse effects on or via lactation

In a Reproduction/Developmental Toxicity Screening Test (OECD 421), described in detail in Chapter 10.12.1. and 10.10.5, some indications of a possible adverse effect on or via lactation were identified.

The substance is lipophilic but no information on a transfer to milk is available; based on the effects seen in pups (postnatal loss, reduced pup weight at highest dose) a transfer can neither be excluded nor approved.

According to the study protocol the stomach of pups not surviving to the scheduled necropsy date were examined for the presence of milk. At 200 mg/kg bw/day most of the pups that were found dead showed absence of milk in the stomach. The five dead pups in the 60 mg/kg bw/day group showed no findings. For seven pups from one litter (#63) in this group at first litter check “no milk” was reported in the protocol, however, it has to be noted that no findings and deads for these animals were documented till the end of the

observation period. At 20 mg/kg bw/day two dead pups from two litters found dead at first litter check showed no milk in the stomach. For the spontaneous death of one pup in the control group no findings are documented (see also Table 19).

Table 19: Mortality and macroscopic findings for pups (Anonymous, 2013b)

Dose group	Litter #	Documentation of macroscopic observation
control	# 42	1m spontaneous death at day 2, no findings
20 mg/kg bw/d	#51	1m dead at first litter check, no milk
	#59	1f dead at first litter check, no milk
60 mg/kg bw/d	#63	For 5m and 2 f no milk at first litter check is documented but pups showed no abnormalities from day 2 to 5 till planed necropsy. 1m and 3f dead at first litter check with no findings
	#66	1f missing on day 3
200 mg/kg bw/d	#71	All pups (4m and 9f) dead at first litter check, no milk
	#73	1m missing on day 6
	#76	1f dead at day 4, no milk 1m missing on day 2
	# 78	1m dead at day 2, no milk 1f missing on day 3
	#79	All pups dead: 3m and 4f dead at first litter check, no milk 1m and 3f dead at day 2, no milk 2m and 1f missing

According to the study description any deficiencies in maternal care (such as inadequate construction or cleaning of the nest, pups left scattered and cold, physical abuse of pups or apparently inadequate lactation or feeding) were examined. No effects on maternal care are reported. Dams of litter #71 and 79 have to be excluded here as they had total litter loss and were euthanized on day 1 or 2 of lactation.

The findings described above for the high and the low dose group, in combination with normal maternal care may indicate that the pups were not able to suckle; this could be an evidence for a direct effect of the test substance on the pups or an indication of developmental toxicity. However, all pups from litter #71 and 79 showed no milk in the stomache and died, therefore, for these litters also an adverse effect on or via lactation or a non-specific secondary effect (maternal toxicity) cannot be excluded.

No comparable effects are documented for the read-across substance EC 404-360-3 in a One-Generation-Reproduction-Toxicity study. Reduced pup weights were seen at the highest dose level of 300 mg/kg bw, however, the pups showed normal body weight gain during lactation. No information on milk in the stomache of dead pups or maternal care is available.

Comparison with the CLP criteria

According to CLP regulation substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the basis of: (a) human evidence indicating a hazard to

babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

Relevant effects seen in a Reproduction/Developmental Toxicity Screening Test with the substance EC 438-340-0 were the absence of milk in pups found dead of the 200 mg/kg bw/day and in the 20 mg/kg bw dose group. The five dead pups in the 60 mg/kg bw/day group showed no such findings. No dose response relationship can be established.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Fertility:

Effects on male reproductive system are described after exposure to 200 mg/kg bw (OECD 421) and 450 mg/kg bw (OECD 407). Effects on epididymides (reduced weight, nodules, cell debris, oligospermia) and testes (reduces weight, small testes, markedly reduced spermatogenesis, slight to marked occurrence of spermatic giant cells, and marked tubular atrophy) are described, even after recovery. However, fertility of male rats was not impaired, but the low sperm reserve in rats versus humans has to be considered. No severe general toxicity in males was described. Based on these findings a classification as Repr. 1B (H360F; May damage fertility) is proposed.

Developmental toxicity:

Foetal toxicity (dead pups at first litter check, postnatal loss, reduced pup weight) is documented in the Reproduction / Developmental Toxicity Screening Test with the substance EC 438-340-0 at a concentration of 200 mg/kg bw and at a lower extent at 60 mg/kg bw without severe maternal toxicity. This is supported by a One-Generation Reproduction Toxicity Study with the target substance EC 404-360-3 showing dose dependant similar effects (increased number of stillborn pups, postnatal loss and reduced pup weight at 100 and 300 mg/kg bw). Therefore a classification as Repr. 1B (H360D; May damage the unborn child) is proposed.

Adverse effects on or via lactation

Some indications of a possible adverse effect on or via lactation were identified in the Reproduction/Developmental Toxicity Screening Test with the substance EC 438-340-0. No information on a transfer of the substance to milk is available. At 200 mg/kg bw/day most of the pups that were found dead showed absence of milk in the stomach. At 20 mg/kg bw/day two dead pups from two litters found dead at first litter check showed no milk in the stomach. At 60 mg/kg bw dead pups showed no milk related findings. Based on this information no final conclusion on possible effects on or via lactation can be drawn. In addition no clear dose-response relationship can be established. There is no robust evidence that the effects on lactation are caused directly by the substance.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The DS presented two studies with Omnirad 379 in rats: a 28-d study and an OECD TG 421 study, both showing testicular effects. The DS noted that no changes in reproductive organs were observed in studies with the read across source substance Omnirad 369 up to

high doses. Still, the DS proposed classification in Category 1B for adverse effects on sexual function and fertility based on the studies with Omnirad 379 itself.

Adverse effects on development

The DS proposed classification for adverse effects on development in Category 1B based on stillborn pups, early postnatal mortality and reduced pup weight in the OECD TG 421 study with Omnirad 379 and in a 1-generation study with the read across source substance Omnirad 369. Classification of Omnirad 369 as Repr. 1B; H360D was agreed by RAC in 2016.

Effects on or via lactation

The DS mentioned that many of the pups that died in the OECD TG 421 study with Omnirad 379 had no milk in the stomach but acknowledged that this finding cannot be unequivocally attributed to lactation as it may as well represent developmental or maternal toxicity. Consequently, they proposed no classification for effects on or via lactation.

Comments received during consultation

Comments were received from four MSCAs.

As to fertility, all four commenting MSCAs pointed out the lack of testicular toxicity in studies with the source substance Omnirad 369. Two MSCAs also mentioned absence of effects on fertility in the OECD TG 421 study with Omnirad 379; one of them preferred Category 2 for fertility and one did not support Category 1B. Of the other two MSCAs, one supported the DS's proposal for Category 1B and one requested discussion.

In response to these comments, the DS clarified that their proposal for Category 1B was based on studies with Omnirad 379 itself, without applying a read across from Omnirad 369. Further, they stated that the absence of effect on fertility index does not decrease the concern because rats have a higher sperm reserve than humans. They retained their initial position of Category 1B for fertility.

Regarding developmental toxicity, two MSCAs supported Category 1B and another MSCA requested discussion on the role of maternal toxicity in the pup mortality. One MSCA proposed no classification for development as they considered the pup mortality secondary to maternal toxicity.

The DS replied that pup mortality could possibly be attributed to maternal toxicity only in one of the dams in the OECD TG 421 study with Omnirad 379. They further referred to the RAC opinion on Omnirad 369 where RAC concluded that the stillbirths and postnatal mortality in the 1-generation study with Omnirad 369 were unlikely to be secondary to maternal toxicity.

One MSCA supported no classification for adverse effects on or via lactation.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

<u>28-d oral study in rats with Omnirad 379 (Anonymous, 2002a)</u>

Wistar Han rats (HanBrl:WIST, 5/sex/group, 7 weeks old at the beginning of treatment) were administered Omnirad 379 in PEG via gavage at dose levels of 0, 15, 50, 150 and 450 mg/kg bw/d. Recovery after 14 days was investigated in an additional group of animals (5/sex/group at 0 and 450 mg/kg bw/d). General toxicity in top dose males included occasional piloerection, reduced body weight (by 11% on day 28) and body weight gain (by 29% days 1-28; 96 g vs 136 g), mild anaemia, hyaline change in renal tubules, and an increase in liver weight (relative, by 29%) associated with hypertrophy and clinical chemistry changes (increased bilirubin, cholesterol and triglycerides).

Top dose males had markedly reduced testes weight (absolute, by 49%; relative, by 43%). Histopathological examination revealed testicular degeneration/atrophy, not reversible by the end of the 2-week recovery period. No testicular effects were found at the next lower dose of 150 mg/kg bw/d.

28-d study in rats with Omnirad 379: testicular findings							
Dose (mg/kg bw/d)	**0**	**15**	**50**	**150**	**450**	**0 recovery**	**450 recovery**
No. of animals per group	5	5	5	5	5	5	5
Initial body weight [a] (g)	199	189	199	193	201		
Body weight gain days 1-28 [a] (%)	68	72	64	60*	48**		
Terminal body weight (g)	297	296	296	279	265**	350	313**
Testes weight, absolute (g)	3.66	3.32	3.33	3.49	1.87**	3.73	2.14**
Testes weight, relative (%)	1.23	1.12	1.12	1.25	0.70**	1.07	0.68**
Testes, tubular atrophy; incidence (mean severity)	0	0	0	0	1 (4.0)	0	4 (2.8)
Testes, spermatic giant cells	0	0	0	0	3 (3.0)	0	1 (1.0)
Testes, reduced spermatogenesis	0	0	0	0	5 (3.8)	0	4 (4.0)
Epididymides weight, absolute (g)	1.06	1.05	1.00	0.96	0.70**	1.26	0.72**
Epididymides weight, relative (%)	0.36	0.36	0.34	0.34	0.27**	0.36	0.23**
Epididymides, cellular debris	0	0	0	0	4 (2.8)	0	5 (1.6)
Epididymides, reduced spermatozoa	0	0	0	0	4 (5.0)	0	4 (5.0)

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

Severity grades: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe

[a] The values in columns "0" and "450" include recovery animals.

OECD TG 421 study in rats with Omnirad 379 (Anonymous, 2013b)

Wistar Han rats (Crl:WI(Han), 10/sex/group, approximately 11 weeks old at the beginning of treatment) were administered Omnirad 379 in PEG via gavage at dose levels of 0, 20, 60 and 200 mg/kg bw/d. Males were treated for 28 days (two weeks prior to mating, throughout mating and until termination), females for 42-52 days (two weeks prior to mating, throughout mating and gestation and until termination after at least 3 days of lactation). Top dose selection was based on a limited 14-d range-finding study in females, where 300 mg/kg bw/d caused clinical signs and body weight loss. The top dose selected for the main study was 200 mg/kg bw/d; top dose males showed reduced body weight (by 7% at termination) and body weight gain (by 45%).

Absolute testes weight was unaffected, absolute epididymides weight was decreased by 17% at 200 mg/kg bw/d. Histopathological examination of the testes showed germ cell exfoliation without degeneration (5 animals minimal, 3 slight, 1 moderate). Cell debris (minimal to moderate) and oligospermia (minimal to slight) were observed in the epididymides. Spermatogenic staging profiles were normal for all males. No reduction in fertility was detected.

OECD TG 421 study with Omnirad 379: parameters related to male fertility				
Dose (mg/kg bw/d)	**0**	**20**	**60**	**200**
No. of animals per group	10	10	10	10
Initial body weight (g)	327	327	326	331
Body weight gain (%)	20	19	15**	11**
Terminal body weight (g)	390	383	371*	362**
Testes weight, absolute (g)	3.64	3.49	3.74	3.45
Testes weight, relative (%)	0.93	0.91	1.01*	0.95
Testes, exfoliating germ cells; incidence (mean severity)	0	0	0	9 (1.6)
Epididymides weight, absolute (g)	1.21	1.13	1.16	1.00**
Epididymides weight, relative (%)	0.31	0.30	0.31	0.28*
Epididymides, cell debris (mean severity)	0	0	0	8 (1.8)
Epididymides, oligospermia (mean severity)	0	0	0	4 (1.3)
Fertility index [a] (%)	80	100	90	100

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

Severity scores: 1 = minimal, 2 = slight, 3 = moderate

[a] females pregnant / females paired x 100

One-generation study in rats with Omnirad 369 (Anonymous, 2011)

Wistar rats (20/sex/group, 5 weeks old at the beginning of treatment) were administered Omnirad 369 in PEG via oral gavage at 0, 30, 100 and 300 mg/kg bw/d. The pre-mating period was at least 74 days. Top dose males showed increased weight of the liver (relative, by 34%) and kidneys with histopathological correlates (hepatocellular hypertrophy, eosinophilic droplets in the kidney) and also increased adrenal weight. Top dose selection was based on a 28-d range-finding study (Anonymous, 2009) where 500 mg/kg bw/d caused excessive toxicity (hunched posture, piloerection, lean appearance, retching, gasping, body weight loss or reduced weight gain).

No histopathological findings were observed in the reproductive organs of top dose males or females. Absolute testes weight was slightly increased (by 7%), prostate weight decreased (absolute and relative by 20% and 15%, respectively). Fertility-related parameters (such as fertility index) showed no significant alterations. Sperm parameters were not investigated.

One-generation study with Omnirad 369: fertility-related parameters					
Dose (mg/kg bw/d)	**0**	**30**	**100**	**300**	**HCD**
Males placed with females	20	20	19	20	
Males that did not mate	0	0	0	0	
Females pregnant	19	19	20 [a]	17	
Females not pregnant	1	1	0	3	
Male fertility index (%)	95	95	100	85	84-100
No. of implantation sites (±SD)	11.6 (±3.3)	10.9 (±3.4)	10.6 (±4.3)	10.6 (±3.8)	
Testes weight, absolute (% of control)	-	98	100	107*	
Testes weight, relative (% of control)	-	103	103	113*	
Epididymides weight, absolute (% of control)	-	97	100	100	
Epididymides weight, relative (% of control)	-	102	102	106	
Prostate weight, absolute (% of control)	-	97	100	80*	
Prostate weight, relative (% of control)	-	102	102	85*	
Seminal vesicle weight, absolute (% of control)	-	97	99	87*	
Seminal vesicle weight, relative (% of control)	-	102	101	92	

* statistically significant difference from control, $p \leq 0.05$

[a] One male of the mid dose group died prior to mating. Therefore, one male of the mid dose group mated with two females of the mid dose group.

HCD = historical control data

Repeated dose studies with Omnirad 369

In a 14-d gavage study in rats (Anonymous, 1989a; 5 animals/sex/group) several females at 3000 and 1000 mg/kg bw/d were killed within several days due to a marked body weight loss. Reproductive organ weights were reportedly unaffected up to 1000 mg/kg bw/d. A decrease in testicular weight at 3000 mg/kg bw/d is mentioned in the registration dossier without further specification (male body weight was reduced by 20% at this dose). A subsequent 28-d study (Anonymous, 1989b; 5 animals/sex/group, 6 weeks old at the beginning of treatment) with a top dose of 500 mg/kg bw/d reported no macroscopic changes in reproductive organs nor alterations in their weight. Histopathological examination of reproductive organs was not performed.

Another 28-d study (Anonymous, 2009; 5 animals/sex/group, 9-10 weeks old at the beginning of treatment), a range-finding study to the 1-generation study (Anonymous, 2011), started with a top dose of 500 mg/kg bw/d but it had to be reduced to 250 mg/kg bw/d due to excessive toxicity (clinical signs, body weight loss). Histopathological examination of testes (including staging of spermatogenesis) and epididymides did not reveal any treatment related effects. There was no significant change in sperm motility, but the sensitivity was not optimal due to a low number of animals and a low control value (55%).

Conclusion on adverse effects on sexual function and fertility

The 28-d study with Omnirad 379 showed marked testicular degeneration/atrophy at 450 mg/kg bw/d in the presence of some but not excessive general toxicity. Only slight testicular effects were observed, and no fertility reduction was detected at 200 mg/kg bw/d in the OECD TG 421 study. Although the top dose in the OECD TG 421 study was lower than that of the 28-d study, 200 mg/kg bw/d still did cause some general toxicity in both sexes.

The 28-d studies and the 1-generation study with the close structural analogue Omnirad 369 did not reveal any effect on fertility index or testicular histopathology up to 300 mg/kg bw/d nor any changes in testes weight up to 500 mg/kg bw/d. No classification for fertility was agreed by RAC for this substance (RAC, 2016).

RAC agrees with the DS that the evidence of testicular toxicity in two studies with Omnirad 379 warrants classification. RAC, however, also notes that no testicular toxicity and no effect on fertility index were observed in the 1-generation study with Omnirad 369. Therefore, in a weight-of-evidence assessment, RAC concluded that **classification in Category 2 for adverse effects on sexual function and fertility is warranted**.

Adverse effects on development

OECD TG 421 study in rats with Omnirad 379 (Anonymous, 2013b)

Wistar Han rats (10/sex/group) were administered Omnirad 379 in PEG via oral gavage at dose levels of 0, 20, 60 and 200 mg/kg bw/d. Females were treated for two weeks prior to mating, throughout mating and gestation and until termination after at least 3 days of

lactation. Transient piloerection and hunched posture after dosing were occasionally observed in several top dose females, food consumption during gestation and lactation was reduced compared to controls.

Two top dose females were sacrificed on lactation days (LD) 1 (dam no. 71) and 2 (dam no. 79) due to total litter loss. Dam no. 71 was lethargic and pale before sacrifice and histopathological examination showed marked glomerular and tubular necrosis of the kidneys; food consumption was comparable to other females of this group. All 13 pups from dam no. 71 were dead at first litter check. Hunched posture was noted in dam no. 79 on the two last days before sacrifice and she had a somewhat lower food consumption towards the end of gestation compared to other females in this group (gestation day (GD) 17-20: 13 g/d, other animals 17-24 g/d). Out of the 14 pups of dam no. 79, 7 were dead at first litter check and the other 7 were dead or missing on the next day. Both dams also showed lymphoid atrophy of the thymus, which may reflect a stress response. RAC agrees with the author of the study report that the total litter loss in dam no. 71 may be a result of maternal toxicity. Involvement of maternal toxicity is possible also for dam no. 79.

Pup mortality was also increased on subsequent days: 12 pups from 4 litters were found dead (6 pups) or missing (6 pups) by termination on PND 5-7 at the top dose compared to a single pup in the control group. Seven of these dead/missing pups were from dam no. 79. Additionally, significant reduction in pup body weights was noted at the top dose on days 1 (-15%) and 4 (-20%) of lactation.

OECD TG 421 study with Omnirad 379: developmental effects					
Dose (mg/kg bw/d)	**0**	**20**	**60**	**200**	**HCD [a]**
No. of pregnant females	8	10	9	10	
No. of females with total litter loss (day of total litter loss)	0	0	0	2 (days 1, 2)	
No. of living pups (no. of litters with living pups) at first litter check	92 (8)	106 (10)	98 (9)	99 (9)	
No. of dead pups (no. of affected litters) at first litter check	0	2 (2)	4 (1)	20 (2)	
Mean no. of dead pups per litter at first litter check (±SD), including dam no. 71 [b]	0.0 (±0.0)	0.2 (±0.4)	0.4 (±1.3)	2.0 (±4.4)	Mean 0.1 (±0.3) Range 0.0-4.0 P95 1.0
No. of dead pups at first litter check, dam no. 71 excluded [b]	0	2 (2)	4 (1)	7 (1)	
Mean no. of dead pups per litter at first litter check, dam no. 71 excluded (±SD)	0.0 (±0.0)	0.2 (±0.4)	0.4 (±1.3)	0.8 (±2.3)	Mean 0.1 (±0.3) Range 0.0-4.0 P95 1.0
No. of dead or missing pups (no. of affected litters) after	1 (1)	0	1 (1)	12* (4)[c]	

the first litter check until termination on LD 5-7					
Mean no. of dead or missing pups per litter after the first litter check until termination (±SD)	0.1 (±0.4)	0.0 (±0.0)	0.1 (±0.3)	1.2 (±2.2)	Mean 0.1 (±0.4) Range 0.0-4.0 P95 1.0
Viability index [d] (%)	99	100	99	88*	Mean 99
Maternal bw on GD 0 (g)	213	213	214	207	
Maternal bw on LD 1	243	247	244	230	
Maternal food consumption during gestation (g/d)	20	19	19	17	
Maternal food consumption during LD 1-4 (g/d) [e]	28	26	26	22*	
Pup weight LD 1 (g)	6.2	6.1	6.1	5.3*	
Pup weight LD 4 (g)	9.2	9.1	9.1	7.4*	

* statistically significant difference from control, $p \leq 0.05$

[a] same laboratory, within 3 years before the current study, around 900 litters

[b] dam with marked renal toxicity and clinical signs (lethargic, pale)

[c] 6 pups found dead, 6 pups missing; 7 of them from 1 litter (dam no. 79)

[d] no. of pups before planned necropsy / no. of pups born alive x 100

[e] the two top dose females with total litter loss excluded

<u>One-generation study in rats with Omnirad 369 (Anonymous, 2011)</u>

Wistar rats (20/sex/group) were administered Omnirad 369 in PEG via oral gavage at 0, 30, 100 and 300 mg/kg bw/d. Top dose females showed reduced body weight (by up to 8%) and food consumption during lactation, liver hypertrophy (relative liver weight increased by 50%), thyroid hypertrophy and a mild increase in relative kidney weight.

The number of stillborn pups at the top dose was increased above the HCD and occurred across multiple litters. Pup viability was significantly decreased between days 0 and 4. Pup weight at the top dose was reduced by 13% and 21% on days 1 and 21 respectively. The increase in stillborn pups and postnatal mortality led to classification of Omnirad 369 in Category 1B (RAC, 2016).

One-generation study with Omnirad 369: developmental effects					
Dose (mg/kg bw/d)	**0**	**30**	**100**	**300**	**HCD**
Females with implantation sites	19	19	20	17	
Implantation sites (±SD)	11.6 (±3.3)	10.9 (±3.4)	10.6 (±4.3)	10.6 (±3.8)	
Post-implantation loss (%)	18	9	20	13	
No. of litters	18	19	17	17	
No. of pups	194	190	190	162	
Litter size	10.8	10.1	11.2	9.2	9.3-12.8

No. of stillborn pups (no. of females with stillborn pups)	0	2 (1)	6 (5)	9 (8)	
% stillborn pups	0	1.0	3.2	5.6	0-4.5
Pups dead on LD 0	0	1	0	4	
Pups dead LD 1-4	0	3	3	18	
Pups dead LD 5-21	0	0	1	1	
Viability index (pups surviving LD 0-4, %)	100	98	98	86*	94-100
Maternal bw GD 0 (g)	218	218	225	209	
Maternal bw LD 0 (g)	248	246	252	232*	
Maternal bw LD 21 (g)	273	274	279	260*	
Pup bw LD 1 (g)	6.3	6.3	5.9	5.5*	
Pup bw LD 21 (g)	46.6	46.3	44.3	36.9*	

* statistically significant difference from control, $p \leq 0.05$

Conclusion on developmental toxicity

The results of the OECD TG 421 study with Omnirad 379 shows an increase in early postnatal mortality at a dose associated with maternal toxicity. The concern is increased by a similar pattern of effects (stillbirths, early postnatal mortality, decreased pup weight) being observed without marked maternal toxicity in the 1-generation study with a closely related substance, Omnirad 369, which was classified by RAC as Repr. 1B; 360D (RAC, 2016).

RAC agrees with the DS's that **classification in Category 1B for adverse effects on development is warranted**, based on pup mortality in a study with the substance itself and on pup mortality in a study with the close structural analogue Omnirad 369.

Effects on or via lactation

Increased early postnatal mortality was observed in the OECD TG 421 study with Omnirad 379 and in the 1-generation study with Omnirad 369. The OECD TG 421 study mentions no milk (in the stomach) as a necropsy observation in a number of pups that died. At the top dose of 200 mg/kg bw/d no milk is listed as necropsy observation for all 20 pups found dead at first litter check (however, at least part of them may have been stillborn) and all 6 pups from 3 litters found dead at later time points. Nevertheless, this finding might be related to either maternal toxicity (in the litters of dams no. 71 and 79, see above) or developmental toxicity. Pup mortality has already been used to justify classification in Category 1B for adverse effects on development. Therefore, RAC **agrees with the DS that no classification for adverse effects on or via lactation is warranted**.

Overall conclusion on reproductive toxicity

RAC proposes classification of Omnirad 379 as **Repr. 1B; 360Df** and agrees with the DS's proposal of no classification for effects on or via lactation.

10.11 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

For evaluation of this endpoint no 90-day study is available, however, a 28-day study, a reproduction/developmental toxicity screening study with exposure up to 52 days and a 14-day dose range finding study are available and presented below.

Table 20: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD 407 GLP Rat, HanBrl:WIST (SPF)	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (TK 11005, CGI 113) 0, 15, 50, 150, 450 mg/kg bw/d 0 and 450 mg/kg bw/day: n= 10 m + 10 f 15, 50, 150 mg/kg bw/day: n= 5 m + 5 f Oral, gavage (5ml/kg bw) Vehicle Polyethylene glycol 7 days/week for 28 days	NOAEL = 50 mg/kg bw Reduces testis size, weight of testes and epididymides and reduced spermatogenesis (associated with spermic giant cells and tubular atrophy) at 450 mg/kg bw Hyaline changes in kidneys of m at 150 and 450 mg/kg bw Fatty atrophy in bone marrow (450 mg/kg bw) Increased splenic extramedullary hematopoetic activity at 50 mg/kg bw and above in m Hematology: test-item related effects in both sexes at 150 and 450 mg/kg bw/day; haemoglobin and haematocrit values decreased at 450 mg/kg bw Increased liver weight in f at 50 mg/kg bw, increased liver and kidney weights in m and f at 150 and 450 mg/kg bw Slight centrilobular hepatocellular hypertrophy in f at 450 mg/kg bw Reduced body weights in m at 150 mg/kg bw and both at 450 mg/kg bw Hunched posture, piloerection in some animals on some days and dark feces in all high dose animals from day 12 onwards	Anonymous (2002a)
14 day dose range finding study Rat, Crl:WI(Han) N=4 f /dose	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one 0, 150, 300 mg/kg bw Oral, gavage (5ml/kg bw) Vehicle Polyethylene	NOAEL < 150mg/kg bw/day <u>150 mg/kg:</u> slight bw loss in 2f, slightly reduced food consumption (d 5-10), increased liver weights in all f, increased weight of adrenal glands in 1f <u>300 mg/kg bw:</u> bw loss in 2f, reduced food consumption (d 1-10), enlarged liver in 4f, enlarged adrenal glands in 2f, increased liver weights in all f, increased weight of adrenal glands in 2f;	Anonymous (2013a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
	glycol Daily, 14 days	hunched posture, piloerection, rales (0-3h after dosing),	
OECD 421 Reproduction / Developmental Toxicity Screening Test GLP Rat, Crl:WI(Han) N=10/sex/group	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one 0, 20, 60, 200 mg/kg bw 28 days (males) 42 - 52 days (females) Oral, gavage Vehicle: polyethylene glycol	NOAEL (systemic, m) = 20 mg/kg bw/day NOAEL (systemic, f) = 60 mg/kg bw <u>200 mg/kg bw/day:</u> Reduced bw gain in m; bw loss in f week 1 of treatment, decreased food consumption in f; Lymphoid atrophy (moderate) of the thymus in 2/10 females Epididymides with reduced weight, cell debris and oligospermia. germ cell exfoliating into the lumen of seminiferous tubules of testes Signif. reduced viability index (87.9%) Dead pups (20) at first litter check from 2/10 females Postnatal loss (-12.1%) in 4/10 litters Reduced pup body weights (-20% on day 4) <u>60 mg/kg bw/day:</u> Reduced body weight gain in m Dead pups (in total 5) from 2/10 females	Anonymous (2013b)

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a **14-day dose range finding study** (Anomynous, 2013a) Crl:WI(Han) female rats were exposed via gavage to dose levels of 0, 150 and 300 mg/kg bw/day. Formulations were prepared daily in propylene glycol and were stable at room temperature and under normal laboratory light conditions for at least 5h. Clinical signs were recorded on all days after dosing (15min, 1h, 3h) and body weights were taken on days 1, 5, 10 and 14. Blood samples were collected on day 15. At necropsy the organ weights of adrenal glands, kidneys, liver and spleen were determined. No statistical analysis was performed due to the low number of animals per group.

No mortalities occured during the study. Clinical signs of toxicity were noted at 300 mg/kg bw/day: hunched posture was noted for all animals on several days, piloerection was noted for all animals on two days and rales were noted for one animal on one day. Dose related effects on body weights and body weight gain were noted (see Table 21). At 150 mg/kg bw/day two females showed a body weight loss of -3 or -4% on day 14, and two females at 300 mg/kg bw/day showed a body weight loss of -10% or -2% on day 14 (of which the latter one showed -11% on day 10). Food consumption was slighty reduced at 150 mg/kg bw (days 5-10) and reduced at 300 mg/kg bw (days 1-10). Haematological parameters were considered not to be affected by treatment. At 150 mg/kg bw/day cholesterol was increased for all animals and glucose concentration was increased for one female (#6). At 300 mg/kg bw/day, total protein and albumin levels were decreased while increased

concentrations were noted for glucose, cholesterol and potassium. Macroscopic examinations showed an enlarged liver and adrenal glands in one female (#6) at 150 mg/kg bw. At 300 mg/kg bw/day, three females showed an enlarged liver and two females showed enlarged adrenal glands. Increased weights of adrenal glands were documented for one female at 150 mg/kg bw/day and two females at 300 mg/kg bw/day. Increased liver weights were seen in both dose levels (all animals) (see Table 22). The kidneys and spleen weights of dosed females were similar to control levels.

Table 21: Body weight [gram], body weight gain [%] and food consumption in females (mean ± St.Dev, n=number of animals) (Anonymous, 2013a).

Day	Control group	150 mg/kg bw/d	300 mg/kg bw/d
Body weight [gram]			
Day 1	210 ± 5.1 n=4	215 ± 7.9 n=4	212 ± 9.4 n=4
Day 5	208 ± 3.1 n=4	210 ± 5.7 n=4	198 ± 4.7 n=4
Day 10	217 ± 3.0 n=4	213 ± 6.6 n=4	198 ± 6.3 n=4
Day 14	216 ± 2.9 n=4	215 ± 3.4 n=4	206 ± 13.8 n=4
Body weight gain [%]			
Day 1	0 ± 0.0 n=4	0 ± 0.0 n=4	0 ± 0.0 n=4
Day 5	-1 ± 1.0 n=4	-2 ± 1,5 n=4	-7 ± 2.4 n=4
Day 10	3 ± 1.9 n=4	-1 ± 1.9 n=4	-6 ± 4.5 n=4
Day 14	3 ± 2.2 n=4	0 ± 4.0 n=4	-3 ± 5.3 n=4
Mean food consumption [gram/animal/day]			
Days 1-5	16	13	9
Days 5-10	13	11	7
Days 10-14	14	14	13

No statistical analysis were performed due to the low number of animals per group.

Table 22: Organ weights [gram], females (mean ± St.Dev, n=number of animals) (Anonymous, 2013a).

Parameter	Control group	150 mg/kg bw/d	300 mg/kg bw/d
Body weight at end of treatment	199 ± 2 n=4	198 ± 5 n=4	191 ± 11 n=4

Liver	4.88 ± 0.25 n=4	5.68 ± 0.45 n=4	6.92 ± 0.51 n=4
Adrenals	0.069 ± 0005 n=4	0.070 ± 0.015 n=4	0.077 ± 0.005 n=4

No statistical analysis were performed due to the low number of animals per group.

In a **28-day study** (Anonymous, 2002a) HanBrl:WIST (SPF) rats were orally exposed (gavage) to concentrations of 0, 15, 50, 150 or 450 mg/kg bw/d of the test substance. 10 males and 10 females were used for the control and the high dose group (including 14 day recovery group), 5 males and 5 females for the remaining groups. The test item formulations were prepared daily and analysed by HPLC. Clinical signs, food consumption and body weights were recorded periodically. Functional observational battery, locomotor activity and grip strength were performed during week 4. Blood samples as well as urine samples were taken at the end of dosing and end of recovery periode. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals.

No mortality was observed until scheduled necropsy. Clinical signs were observed in males and females at 450 mg/kg/day only: While piloerection (slight severity) was noted from days 8-14 in several rats, hunched posture (slight) was noted in some females on days 9-12. In females also emaciation (on days 8-10) and waggling gait (days 9-12) was observed. Reversibility was documented. In the highest dose group also dark feces and pale feces were noted in rats from days 12-22 and from days 24-28 of treatment, respectively. The latter finding persisted for two days of the recovery period. These findings were considered likely to be related to the compensated anemia seen in the hematology parameters. Salivation was noted occasionally in rats at 450 mg/kg/day during the treatment period.

During the functional observational battery no clinical signs of neurological impairment (grip strength, locomotor activity) were documented.

The mean body weights were reduced in both sexes treated with 450 mg/kg/day from treatment day 8 onwards when compared with the control rats. On day 28 a reduction of -11.3% and -13.8% in males and females, respectively, is documented. During the recovery period, the mean body weights of rats in this dose group remained lower than those of the controls, although the mean body weight gain improved. In males treated with 150 mg/kg/day a reduction was seen from day 22 onwards with a reduction of -7.8% on treatment day 28. The mean body weights of females treated with 150 mg/kg/day were unaffected.

The absolute mean daily food consumption was reduced during the first measurement interval (days 1-8) in males and females at 450 mg/kg/day when compared with controls, but improved thereafter to levels comparable to that of the controls. This trend was also seen in the relative food consumption. The mean daily food consumption of the remaining groups was unaffected.

Table 23: Mean body weight [gram] and mean body weight gain [%] of males and females in gram (mean ± St.Dev, n=number of animals, % bw gain) (Anonymous, 2002a).

Day	Control group	15 mg/kg bw/d	50 mg/kg bw/d	150 mg/kg bw/d	450 mg/kg bw/d
males					
Day 1	199 ± 7.4 n=10 -	189 ± 5.0 n=5 -	199 ± 4.6 n=5 -	193 ± 8.7 n=5 -	201 ± 8.9 n=10 -
Day 8	240 ± 10.2 n=10 20.5%	230 ± 8.6 n=5 21.6%	239 ± 6.4 n=5 19.9%	230 ± 10.6 n=5 19.0%	219** ± 14 n=10 8.9%**

Day 15	278 ± 15.6 n=10 39.3%	269 ± 6.4 n=5 42.3%	275 ± 8.0 n=5 37.7%	265 ± 12.1 n=5 37.2%	249** ± 11.8 n=10 24.0%**
Day 22	308 ± 20.0 n=10 54.6%	301 ± 7.5 n=5 59.1%	305 ± 10.5 n=5 53.1%	289 ± 14.5 n=5 49.6%	274** ± 12.2 n=10 36.3%**
Day 28	335 ± 24.7 n=10 68.2%	326 ± 6.2 n=5 72.4%	327 ± 13.0 n=5 64.1%	309* ± 16.9 n=5 60.2%*	297** ± 13.1 n=10 48.1%**
Recovery day 1	324 ± 18.7 n=5 60.6%	-	-	-	278** ± 13.9 n=5 40.1%**
Recovery day 8	360 ± 24.7 n=5 78.4%	-	-	-	314* ± 17.9 n=5 57.7%
Recovery day 14	379 ± 30.9 n=5 87.6%	-	-	-	338 ± 21.9 n=5 69.9%
females					
Day 1	143 ± 10.2 n=10 -	137 ± 6.9 n=5 -	141 ± 13.3 n=5 -	142 ± 8.5 n=5 -	141 ± 12.1 n=10 -
Day 8	163 ± 9.7 n=10 13.9%	155 ± 8.1 n=5 13.3%	162 ± 12.4 n=5 15.0%	158 ± 6.6 n=5 12.0%	135** ± 9.7 n=10 -4.2%**
Day 15	176 ± 11.1 n=10 23.3%	169 ± 8.4 n=5 23.8%	179 ± 14.6 n=5 27.2%	174 ± 7.6 n=5 23.3%	159** ± 12.1 n=10 13.1%**
Day 22	190 ± 12.5 n=10 32.8%	182 ± 11.7 n=4 33.5%	193 ± 16.6 n=5 37.1%	185 ± 7.5 n=5 30.7%	167** ± 13.8 n=10 18.7%**
Day 28	203 ± 13.2 n=10 41.7%	191 ± 11.4 n=5 39.3%	203 ± 15.9 n=5 44.7%	195 ± 10.6 n=5 37.8%	175** ± 14.7 n=10 23.8%**
Recovery day 1	185 ± 11.8 n=5 28.0%	-	-	-	155** ± 5.0 n=5 16.0%*
Recovery day 8	203 ± 11.0 n=5	-	-	-	177* ± 9.3 n=5

	40.5%				32.3%*
Recovery day 14	212 ± 13.8 n=5 46.8%	-	-	-	186* ± 8.3 n=5 38.8%

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Hematology showed some test-item related effects in both sexes at 150 and 450 mg/kg bw/day (Table 24). In females the red blood cell count was reduced at 50 mg/kg/day ($p<0.05$), 150 mg/kg/day ($p<0.05$) and 450 mg/kg/day ($p<0.01$). The absolute and relative reticulocyte counts were significantly increased (both $p<0.01$) in the males treated with 450 mg/kg/day (compensatory reticulocytosis). Although a slight increase in these parameters was noted in males treated with 150 mg/kg/day, only the absolute reticulocyte count attained statistical significance ($p<0.05$). A shift toward significantly increased number of high fluoresence reticulocytes was noted in males treated with 450 mg/kg/day (13.6% compared to control with 8.6%). The platelet count was significantly higher in males treated with 150 mg/kg/day ($p<0.05$) and 450 mg/kg/day ($p<0.01$). Hemoglobin ($p<0.01$) and hematocrit ($p<0.01$) were reduced in both sexes at 450 mg/kg/day and hematocrit ($p<0.05$) was reduced in females treated with 150 mg/kg/day. Corresponding changes in the mean cell hemoglobin ($p<0.01$), mean cell hemoglobin concentration ($p<0.05$) and mean corpuscular volume ($p<0.01$) were noted in the males treated with 450 mg/kg/day. Increased leukocyte counts in males at 150 mg/kg/day ($p<0.05$) and both sexes at 450 mg/kg/day ($p<0.01$) reflected higher absolute segmented neutrophil counts in males at 150 mg/kg/day and 450 mg/kg/day and higher absolute lymphocyte counts in both sexes at 450 mg/kg/day. After the two week recovery period, the red blood cell count of the females previously treated with 450 mg/kg/day remained lower compared to the control animals. In high dosed males the mean cell hemoglobin concentration remained lower, the absolute and relative reticulocyte counts higher and the left shift in the reticulocyte fluorescence ratios persisted. All other differences noted after the two-week recovery period when compared to the control values were considered to be incidental by the study authors.

Table 24: Selected hematology parameters for males and females (Anonymous, 2002a).

Parameter [unit]	Control group	15 mg/kg bw/d	50 mg/kg bw/d	150 mg/kg bw/d	450 mg/kg bw/d	Control recovery	450 mg/kg bw/d recovery
males							
RBC [T/L]	8.49	8.41	8.68	8.70	8.58	8.37	8.18
HB [mmol/L]	9.95	9.82	10.23	9.82	9.51**	9.81	9.48
HCT [L/L]	0.47	0.46	0.48	0.46	0.45**	0.47	0.47
MCV [fL]	56.0	55.1	55.2	53.0*	52.0**	56.4	57.1
MCH [fmol]	1.17	1.17	1.18	1.13	1.11**	1.17	1.16
MCHC [mmol/L]	20.9	21.2	21.4*	21.3	21.3*	20.8	20.3**
PLATELETS [G/L]	1016	1143	1143	1220*	1275**	1008	1122
Reticulocyte [%]	3.01	3.25	3.11	3.45	3.79**	3.29	4.47**

WBC [G/L]	6.5	5.8	6.7	8.5*	10.0**	7.1	5.9
females							
RBC [T/L]	8.07	7.81	7.68*	7.67*	7.32**	7.89	7.48*
HB [mmol/L]	9.38	9.18	9.15	9.15	8.57**	9.78	9.45
HCT [L/L]	0.45	0.44	0.43	0.43*	0.41**	0.47	0.46
MCV [fL]	55.6	56.5	56.4	56.2	55.9	59.9	61.9
MCH [fmol]	1.16	1.18	1.19	1.19	1.17	1.24	1.26
MCHC [mmol/L]	20.9	20.8	21.1	21.2	20.9	20.7	20.4
PLATELETS [G/L]	1088	970	985	978	1072	1031	1059
Reticulocyte [%]	3.29	3.42	3.47	3.36	3.79	3.32	3.74
WBC [G/L]	4.2	5.2	4.1	5.5	6.2**	4.8	5.0

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

Erythrocyte count RBC, hemoglobin HB, hematocrit HCT, mean corpuscular volume MCV, mean corpuscular hemoglobin MCH, mean corpuscular hemoglobin concentration MCHC, platelet count PLATELETS, total leukocyte count WBC

Clinical biochemistry showed the following test item-related changes in rats treated at 450 mg/kg/day: increased creatinine (males $p<0.01$), total bilirubin (males $p<0.01$), total cholesterol (both sexes $p<0.01$), triglycerides (both sexes $p<0.01$), phospholipids (both sexes $p<0.01$), albumin (males $p<0.01$), total protein (males $p<0.01$) and calcium levels (males $p<0.01$, females $p<0.05$) compared to the controls. Reduced aspartate aminotransferase activity in males treated with 150 mg/kg/day ($p<0.01$) and 450 mg/kg/day ($p<0.01$) was not supported by changes in related parameters and therefore considered unlikely to be a test item-related effect. Gamma glutamyl transferase activity was about 5 times higher in females at 450 mg/kg/day compared to control ($p<0.01$). These findings were considered to be related to the test item and probably indicative of minor changes in liver metabolism. The elevated albumin and total protein levels in males treated with 450 mg/kg/day persisted after the two-week recovery period. Also elevated gamma glutamyl transferase activity noted in high dosed females was irreversible in the 14-day observation period.

No test item-related changes in the urinalysis parameters were noted at the end of treatment.

Examination of male organ weights after four weeks of treatment with 450 mg/kg/day showed test item related changes in absolute organ weights and organ-to-brain weight ratios in the livers (+15% $p<0.05$,), kidneys (+13%), testes (-49%, $p<0.01$) and epididymides (-34%, $p<0.01$) of rats.

In females, test item-related changes in absolute organ weights were noted as increased liver weights (+42%, $p<0.01$). A similar change was noted in the liver-to-brain weight ratio. Increased absolute ovary weights (+23%) and ovary-to-body weight ratios ($p<0.05$), noted in females at 450 mg/kg/day, were considered likely to represent normal variations due to estrus, as there were no microscopical alterations which correlated to this finding.

Macroscopic observations show small testes ("diminished in size") in 5/5 males at 450 mg/kg/day at the end of treatment and in 4/5 males at the end of the two week recovery period. No testes findings in lower dose groups. The remaining macroscopic findings were considered to be typical background findings unrelated to the treatment with the test item.

Table 25: Organ weights [gram] for males and females after 28 days of treatment and recovery (mean ± St.Dev, n=5 for all parameters) (Anonymous, 2002a).

Parameter [unit]	Control group	15 mg/kg bw/d	50 mg/kg bw/d	150 mg/kg bw/d	450 mg/kg bw/d	Control recovery	450 mg/kg bw/d recovery
males							
Body weight	296.802 ± 22.420	296.018 ± 9.262	296.316 ± 11.155	278.752 ±15.524	264.908** ± 11.481	349.948 ± 27.066	312.664** ± 17.685
Brain	1.95 ± 0.08	1.97 ± 0.07	1.96 ± 0.05	2.01 ± 0.08	1.94 ± 0.07	2.05 ± 0.08	1.93* ± 0.05
Liver	8.59 ± 0.88	8.66 ± 0.32	8.67 ± 0.67	9.17 ± 0.82	9.89* ± 0.75	9.35 ± 1.07	9.45 ± 0.21
Kidney	2.02 ± 0.25	2.14 ± 0.20	2.08 ± 0.13	2.20 ± 0.16	2.30 ± 0.11	2.39 ± 0.33	2.22 ± 0.19
Testes	3.66 ± 0.39	3.32 ± 0.17	3.33 ± 0.29	3.49 ± 0.35	1.87** ± 0.42	3.73 ± 0.19	2.14**±0.97
Epididymides	1.062 ± 0.116	1.051 ± 0.122	1.001 ± 0.034	0.959 ± 0.033	0.704** ± 0.102	1.260 ± 0.099	0.717** ± 0.153
females							
Body weight	185.054 ± 12.866	173.258 ± 8.302	182.502 ± 17.054	174.978 ± 8.459	162.966* ± 13.367	192.306 ± 12.776	172.984* ± 9.557
Brain	1.85 ± 0.08	1.79 ± 0.08	1.73 ± 0.04	1.81 ± 0.07	1.77 ± 0.09	1.83 ± 0.04	1.76* ± 0.05
Liver	5.52 ± 0.58	5.73 ± 0.56	6.07 ± 0.48	5.98 ± 0.44	7.85** ± 0.73	5.65 ± 0.62	5.66 ± 0.38
Kidney	1.30 ± 0.09	1.37 ± 0.20	1.43 ± 0.07	1.41 ± 0.12	1.43 ± 0.12	1.35 ± 0.14	1.25 ± 0.04
Ovary	0.101 ± 0.022	0.119 ± 0.027	0.095 ± 0.020	0.098 ± 0.019	0.124 ± 0.023	0.113 ± 0.015	0.130 ± 0.023

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

Microscopic examination demonstrated that the test substance produced morphological alterations in the male genital system, male kidneys, male spleen and male/female bone marrow, and female liver and lung:

- The testes of all 5 males at 450 mg/kg/day exhibited moderately to markedly reduced spermatogenesis, three of five males had slight to marked occurrence of spermatic giant cells, and one male had marked tubular atrophy. At the end of recovery period, testes in four of five males at 450 mg/kg/day still were affected and exhibited markedly reduced spermatogenesis and slight to marked tubular atrophy. In one of these individuals, minimal occurrence of spermatic giant cells was observed. At the end of treatment high dosed males showed epididymides containing cellular debris (slight ot marked) and severely reduced amount of spermatozoa. These changes were considered to be secondary to the effect on testes, coincided in three males, whereas in one additional male only cellular debris and in another male only reduced spermatozoa were observed. Following the recovery period, minimal to slight cellular debris occurred in all five males and severely reduced spermatozoa were recorded in four of five individuals at 450 mg/kg/day. The findings in the male genital system correlated with macroscopic observation of small testes, as well as with reductions in organ weight of testes and epididymides.
- The kidneys of males at 150 and 450 mg/kg/day exhibited increased occurrence of tubular hyaline change, probably represented accumulation of alpha2-u globulin, specific to male rats. Following the recovery period, this change was still slightly more prominent at 450 mg/kg/day compared to the controls.
- In the bone marrow at the end of the treatment period, fatty atrophy occurred with minimally increased incidence and grading in males and females at 450 mg/kg/day when compared to controls. This effect was reversible.

- Splenic extramedullary hematopoietic activity was increased in males at 50, 150 and 450 mg/kg/day (with mean grades of 2.6, 3.0 and 3.4 respectively compared to 2.2 in control) and remained higher at the end of recovery (mean grade 3.8). These changes in hematopoietic tissues were accompanied by decreased hemoglobin and hematocrit values and increased proportion of reticulocytes in the peripheral blood, especially in males, and they probably reflect mild effects on the erythropoietic activity.
- Slight centrilobular hepatocellular hypertrophy occurred in several females at 450 mg/kg/day (but no changes in liver enzymes except slightly increased gamma glutamyl transferase activity), and the presence of pulmonary alveolar foam cells was slightly increased in females at 450 mg/kg/day. This lung finding was still slightly prominent after recovery.

It can be concluded that effects seen at 50 mg/kg bw can be considered as non adverse. Clear adverse effects on body weight (m, f), food consumption, male genital system (small testes, reduced weights of testes and epididymides, reduced spermatogenesis), liver (increased weight in m and f), kidney (hyaline changes in m) and the hematopeotic system (decreased hemoglobin and hematocrit values in m and f, increased reticulocytes in m) were seen at 450 mg/kg bw and in a minor extend at 150 mg/kg bw. A NOAEL of 50 mg/kg bw can be derived based on the reported results.

In a **Reproduction / Developmental Toxicity Screening Study** (Anonymous, 2013b) Crl:WI(Han) rats were exposed (oral, gavage) to 0, 20, 60 or 200 mg/kg bw/day. Males were exposed for 28 days (two weeks prior to mating, mating, up to termination), females for 42-52 days (two weeks prior to mating, mating, during post-coitum, at least 3 days of lactation). Formulations were prepared daily in propylene glycol and were stable at room temperature and under normal laboratory light conditions for at least 5h. The following observations and examinations were evaluated: mortality/viability, clinical signs (daily), body weight and food consumption (at least at weekly intervals), macroscopy at termination, organ weights and histopathology on a selection of tissues, reproduction/developmental parameters consisting of mating, fertility and conception indices, precoital time, number of corpora lutea and implantation sites, gestation index and duration, parturition, maternal care, sex ratio and early postnatal pup development (mortality, clinical signs, body weights and macroscopy).

No mortality occurred during the study period in parental animals. At 200 mg/kg bw/day, two females (#71 and 79) were euthanized on day 1 or 2 of lactation as they showed a total litter loss. General clinical signs (1 to 2 hours after dosing) at 200 mg/kg bw were piloerection (6f), hunched posture (3f) observed on some days during premating and in week 3 and 4 after mating. One high dosed female (#71) also showed lethargy and pale appearance on a single day. Salivation was seen after dosing at 60 and 200 mg/kg bw/day in week four after mating. At the mid and highest dose reduced body weight gain was noted for males; decreased bw was only reported for the highest dose. Females showed bw loss during the first week of treatment (but recovered during the remainder of the study) (see Table 26) which was accompanied by a decreased food consumption. At 200 mg/kg bw during lactation also decreased food consumption was reported (Table 28). In males food consumption was not affected.

Table 26: Body weight of males and females in gram (mean ± St.Dev, n=number of animals) (Anonymous, 2013b).

Day	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
males				
Premating Day 1	327 ± 8.1 n=10	327 ± 12.0 n=10	326 ± 5.8 n=10	331 ± 7.6 n=10
Premating Day 8	348 ± 10.8 n=10	346 ± 13.0 n=10	340 ± 7.6 n=10	335* ± 9.2 n=10
Mating Day 1	366 ± 13.5 n=10	362 ± 13.5 n=10	353 ± 8.7 n=10	346** ± 10.2 n=10
Mating Day 8	375 ± 15.9 n=10	374 ± 15.0 n=10	362 ± 8.1 n=10	353** ± 12.2 n=10

Mating Day 15	392 ± 16.5 n=10	389 ± 17.7 n=10	376 ± 8.5 n=10	367** ± 15.7 n=10
females				
Premating Day 1	201 ± 7.1 n=10	204 ± 9.0 n=10	202 ± 5.7 n=10	204 ± 10.3 n=10
Premating Day 8	206 ± 10.4 n=10	203 ± 6.9 n=10	204 ± 8.3 n=10	196 ± 10.0 n=10
Mating Day 1	211 ± 7.4 n=10	213 ± 8.1 n=10	213 ± 9.5 n=10	211 ± 7.4 n=10
Mating Day 8	231 n=1	-	226 n=1	-
Mating Day 15	249 n=1	-	-	-
Mating Day 22	238 n=1	-	-	-
Mating Day 29	240 n=1	-	-	-
Post coitum Day 0	213 ± 10.9 n=8	213 ± 7.8 n=10	214 ± 9.3 n=9	207 ± 7.7 n=10
Post coitum Day 4	225 ± 10.2 n=8	227 ± 9.2 n=10	226 ± 7.0 n=9	220 ± 8.5 n=10
Post coitum Day 7	233 ± 8.6 n=8	235 ± 11.0 n=10	236 ± 6.1 n=9	229 ± 8.7 n=10
Post coitum Day 11	248 ± 13.2 n=8	248 ± 12.6 n=10	249 ± 7.3 n=9	240 ± 7.3 n=10
Post coitum Day 14	261 ± 11.1 n=8	263 ± 12.1 n=10	261 ± 7.0 n=9	254 ± 9.3 n=10
Post coitum Day 17	283 ± 14.8 n=8	285 ± 9.5 n=10	284 ± 8.3 n=9	274 ± 9.3 n=10
Post coitum Day 20	319 ± 21.0 n=8	317 ± 12.1 n=10	315 ± 13.1 n=9	305 ± 16.3 n=10
Lactation Day 1	243 ± 14.3 n=8	247 ± 12.1 n=10	244 ± 11.6 n=9	230 ± 12.4 n=10
Lactation Day 4	261 ± 15.2 n=8	260 ± 11.6 n=10	257 ± 6.5 n=9	252 ± 8.3 n=8

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Table 27: Body weight gain [%] of males and females (mean ± St.Dev, n=number of animals) (Anonymous, 2013b).

Day	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
males				
Premating Day 1	0 ± 0.0 n=10	0 ± 0.0 n=10	0 ± 0.0 n=10	0 ± 0.0 n=10
Premating Day 8	6 ± 1.5 n=10	6 ± 0.6 n=10	4** ± 1.2 n=10	1** ± 1.6 n=10
Mating Day 1	12 ± 3.8 n=10	11 ± 1.3 n=10	9** ± 1.7 n=10	4** ± 2.3 n=10
Mating Day 8	15 ± 3.5 n=10	14 ± 1.8 n=10	11* ± 1.4 n=10	7** ± 3.2 n=10
Mating Day 15	20 ± 3.8 n=10	19 ± 2.3 n=10	15** ± 1.6 n=10	11** ± 4.2 n=10
females				
Premating Day 1	0 ± 0.0 n=10	0 ± 0.0 n=10	0 ± 0.0 n=10	0 ± 0.0 n=10
Premating Day 8	3 ± 2.3 n=10	0 ± 2.9 n=10	1 ± 3.4 n=10	-4** ± 4.9 n=10
Mating Day 1	5 ± 3.0 n=10	5 ± 3.6 n=10	5 ± 3.7 n=10	3 ± 3.8 n=10
Mating Day 8	13 n=1	-	12 n=1	-
Mating Day 15	21 n=1	-	-	-
Mating Day 22	16 n=1	-	-	-
Mating Day 29	17 n=1	-	-	-
Post coitum Day 0	0 ± 0.0 n=8	0 ± 0.0 n=10	0 ± 0.0 n=9	0 ± 0.0 n=10
Post coitum Day 4	6 ± 1.7 n=8	7 ± 2.0 n=10	6 ± 2.7 n=9	6 ± 2.7 n=10
Post coitum Day 7	10 ± 2.6 n=8	10 ± 3.0 n=10	11 ± 4.0 n=9	11 ± 3.4 n=10
Post coitum Day 11	17 ± 2.4 n=8	16 ± 3.9 n=10	17 ± 5.3 n=9	16 ± 4.1 n=10
Post coitum	22 ± 3.7	24 ± 4.1	23 ± 5.7	23 ± 5.4

Day 14	n=8	n=10	n=9	n=10
Post coitum Day 17	33 ± 5.0 n=8	34 ± 4.1 n=10	33 ± 6.5 n=9	33 ± 5.7 n=10
Post coitum Day 20	50 ± 7.9 n=8	49 ± 5.7 n=10	48 ± 8.6 n=9	48 ±8.5 n=10
Lactation Day 1	0 ± 0.0 n=8	0 ± 0.0 n=10	0 ± 0.0 n=9	0 ± 0.0 n=10
Lactation Day 4	7 ± 4.1 n=8	5 ± 1.9 n=10	6 ± 4.6 n=9	8 ± 1.9 n=8

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Table 28: Food consumption in females [gram/animal/day] (mean ± St.Dev, n=number of animals) (Anonymous, 2013b).

Day	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
females				
Premating Days 1-8	14 ± 0.5 n (cage) = 2	14 ± 0.5 n (cage) = 2	14 ± 0.1 n (cage) = 2	10 ± 0.5 n (cage) = 2
Premating Days 8-15	14 ± 0.4 n (cage) = 2	15 ± 0.5 n (cage) = 2	14 ± 0.2 n (cage) = 2	14 ± 1.5 n (cage) = 2
Post coitum Days 0-4	16 ± 4.0 n=8	15 ± 1.8 n=10	15 ± 1.8 n=9	14 ± 2.0 n=10
Post coitum Days 4-7	18 ± 1.4 n=8	18 ± 2.0 n=10	18 ± 1.6 n=9	16 ± 1.8 n=10
Post coitum Days 7-11	19 ± 1.8 n=8	18 ± 2.0 n=10	19 ± 2.0 n=9	17 ± 2.0 n=10
Post coitum Days 11-14	21 ± 4.3 n=8	20 ± 1.7 n=10	20 ± 2.3 n=9	18* ± 1.5 n=10
Post coitum Days 14-17	22 ± 4.0 n=8	20 ± 1.7 n=10	20 ± 2.4 n=9	19* ± 1.9 n=10
Post coitum Days 17-20	22 ± 2.4 n=8	22 ± 1.9 n=10	21 ± 3.0 n=9	20 ± 3.1 n=10
Lactation Days 1-4	28 ± 4.9 n=8	26 ± 1.5 n=10	26 ± 5.0 n=9	22* ± 2.5 n=8

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Macroscopic examination of males showed nodule at the epididymides and enlarged mandibular lymph nodes in some animals without dose response. In females alopecia was seen in singular animals and focus/foci on the clitoral glands were seen in one control female (1/10) and two high dose females (2/8). These findings were reported to be within the background range of findings that are encountered among rats of this age and strain. For the two females that showed a total litter loss at 200 mg/kg bw (necropsy was within 24h) macroscopic

findings in kidneys and/or thymus are reported. Female #71 showed general pale discolouration, many reddish foci on the kidneys and gelatinous thymus. Female #79 showed the thymus reduced in size (see Table 29).

Table 29: Individual body weights and macroscopic findings of females of high dose group (200 mg/g bw) (Anonymous, 2013b).

Animal #	Body weight [mg/kg bw]				Macroscopic findings after dosing with 200 mg/kg bw
	Premating day 1	Premating day 8	Mating day 1	Post coitum day 0	
71§	190	187	206	202	Total litter loss; whole body: discolouration, pale.; Kidney: Both sides: focus/foci, many, reddish; thymus: Gelatinous.
72	206	181	214	203	No findings noted
73§	212	194	218	217	No findings noted
74	188	200	208	203	Clitoral glands – right side: focus/foci, isolated
75	208	197	211	208	Clitoral glands - right side: focus/foci, d=6x3 mm
76§	213	211	216	217	No findings noted
77	212	200	206	197	No findings noted
78§	193	186	195	197	No findings noted
79§	204	194	211	210	Total litter loss; thymus: reduced in size
80	216	211	221	215	No findings noted

§ animals with postnatal loss

Only organ weights of testes and epididymides were examined (Table 30). For males terminal body weights were decreased at 60 and 200 mg/kg bw/day. Absolute and relative weight of the epididymides was statistically significantly reduced at 200 mg/kg bw/day (-17%). A slightly higher mean testes weight in the mid dose group resulted in a statistically significant higher organ/body weight ratio (control 0.93±0.05 versus 60 mg/kg bw 1.01*±0.06). No dose response for this effect was observed.

Table 30: Organ weights [gram], males (mean ± St.Dev, n=number of animals) (Anonymous, 2013b).

Parameter	Control group	20 mg/kg bw/d	60 mg/kg bw/d	200 mg/kg bw/d
males				
Body weight at end of treatment	390 ± 16 n=10	383 ± 18 n=10	371* ± 9 n=10	362** ± 14 n=10
Testes	3.64 ± 0.21 n=10	3.49 ± 0.25 n=10	3.74 ± 0.22 n=10	3.45 ± 0.19 n=10
Epididymides	1.206 ± 0.119 n=10	1.130 ± 0.102 n=10	1.158 ± 0.098 n=10	0.998** ± 0.124 n=10

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Males treated with 200 mg/kg bw showed the following microscopic findings in testes and epididymides: intraluminal cell debris of the epididymides in 8/10 males (up to moderate degree), oligospermia of the epididymides in 4/10 males (up to slight degree), germ cell exfoliating into the lumen of seminiferous tubules

of the testes (without degeneration) in 9/10 males (up to moderate degree). However, no impaired fertility has been seen (Chapter 10.10.2, Table 9); this may be due to the higher sperm reserve in rats versus humans (Mangelsdorf, 2003).

For females treated with 200 mg/kg bw findings in the kidneys and thymus were recorded: glomerular and tubular necrosis (marked) of the kidneys in 1/10 females (noted at necropsy as many reddish foci, both sides), lymphoid atrophy (moderate) of the thymus in 2/10 females (#71 and 79), noted at necropsy as gelatinous or reduced size.

Hematology was not investigated.

In males clear substance related effects were seen at 200 mg/kg bw/day with reduced body weight (gain) in males, epididymides with reduced weight and microscopic findings in testes and epididymides. At 60 mg/kg bw/day only effects on body weight gain in males were seen. In high dose females some effects on food consumption and bw gain were documented but no clear trend over time. Two females showed effects on thymus and one on kidney. A NOAEL of 20 mg/kg bw can be derived for males and a NOAEL of 60 mg/kg bw for females.

For discussion of toxic effects on fertility see Chapter 10.10.2. Developmental parameters examined in the screening study are presented in Chapter 10.10.5.

10.12.2 Comparison with the CLP criteria

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity to humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from studies in animals that cause such effects at or below given Guidance Values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: • reliable and good quality evidence from human cases or epidemiological studies; or • observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided in the CLP regulation in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2

The guidance values for classification as STOT RE (oral exposure) are as follows (CLP-guidance document 3.9.2.2, Haber's rule):

Rat [mg/kg bw]	90 day	28d	49d
Category 1	$C \leq 10$	$C \leq 30$	$C \leq 16$

Category 2	10 < C ≤100	30 < C ≤ 300	16 < C ≤ 163

In the available 28-day study at 450 mg/kg bw/d effects on testes and epididymidis (impairment of fertility) have been reported as well as effects on body weight (↓ m,f) and liver weight (↑ m, f). They are accompanied by changes of hematology parameters described at 150 mg/kg bw/d and above, however, most of them in a range of +/- 9%. Only platelet count, reticulocyte count and total leukocyte count in males reached values around +25, +25 and +53%, respectively. In addition splenic extramedullary hematopoietic activity were seen in males. For females a leukocyte count of +47% is reported for the highes dose group.

In the 14-day study (females only) hematology parameters have not been affected at concentrations up to 300 mg/kg bw/day but liver weight was increased about 42%. No findings in spleen.

In the Repr/Dev screening study hematology has not been investigated as well as spleen and liver. At 200 mg/kg bw effects on body weight, epididymidis weight (-17%), microscopic changes in testes and epididymidis as well as effects on thymus/kidney in 2 females have been documented. Based on reduced bw and slights developmental toxicity at 60 mg/kg bw a NOAEL of 20 mg/kg bw for systemic toxicity can be derived.

In conclusion, the indications of a slight substance related anemia and its compensatory effects seen are not regarded as relevant for classification as STOT RE.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on available subchronic data and the therein documented slight substance related effects on hematology, liver and spleen no classification for STOT RE is proposed.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The dataset for Omnirad 379 comprises two short-term oral studies in rats: a 28-d study according to OECD TG 407 and a Reproduction/Developmental Toxicity Screening Test according to OECD TG 421, both according to GLP. The DS briefly discussed effects on haematology, liver and spleen. They did not consider the observed effects sufficient for classification.

Comments received during consultation

Comments were received from three Member State Competent Authorities (MSCAs), all in support of no classification.

Assessment and comparison with the classification criteria

<u>28-d oral study in rats with Omnirad 379 (Anonymous, 2002a)</u>

Wistar Han rats (HanBrl:WIST, 5/sex/group) were administered Omnirad 379 in polyethylene glycol (PEG) via oral gavage at dose levels of 0, 15, 50, 150 and 450 mg/kg bw/d. Recovery after 14 days was investigated in an additional cohort of animals

(5/sex/group at 0 and 450 mg/kg bw/d). The effects at the top dose included reduced body weight (by 11%/14% in males (m)/females (f)), increased liver weight (relative, by 29%/61% in m/f), renal tubular hyaline change in males and a slight anaemia (haemoglobin reduction < 10%). These effects occurred above the extrapolated guidance value for a 28-d study (300 mg/kg bw/d) and are therefore not considered relevant for classification. Changes in male reproductive organs are presented under reproductive toxicity.

Effects at the next lower dose of 150 mg/kg bw/d included increased liver weight (relative, by 16%/15% in m/f) and increased severity of hyaline change in males (no histopathological changes in the kidneys of females). The increase in liver weight at this dose was relatively mild and not accompanied by histopathological findings or clinical chemistry changes. Kidney findings are presented in the table below. Hyaline change in male rats may be related to accumulation of alpha$_{2u}$-globulin, which is a rodent-specific phenomenon. Although special staining was not performed, the occurrence in males only represents a strong indication in this direction.

28-d study in rats with Omnirad 379: kidney findings in males							
Dose (mg/kg bw/d)	**0**	**15**	**50**	**150**	**450**	**0 recovery**	**450 recovery**
Terminal body weight (g)	297	296	296	279	265**	350	313**
Kidney weight, absolute (g)	2.02	2.14	2.08	2.20	2.30	2.39	2.22
Kidney weight, relative (%)	0.68	0.72	0.70	0.79**	0.87**	0.69	0.71
Kidney, tubular hyaline change; incidence (mean severity)	4 (1.0)	3 (1.3)	4 (2.0)	5 (2.8)	5 (3.4)	2 (2.0)	5 (2.4)

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

Severity grades: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe

OECD TG 421 study in rats with Omnirad 379 (Anonymous, 2013b)

Wistar Han rats (Crl:WI(Han), 10/sex/group) were administered Omnirad 379 in PEG via oral gavage at dose levels of 0, 20, 60 and 200 mg/kg bw/d. Males were exposed for 28 days, females for 42-52 days. Toxic effects at the top dose included clinical signs (e.g.; piloerection), a mild body weight reduction (< 10%), changes in testes and epididymides (presented under reproductive toxicity), kidney toxicity (marked glomerular and tubular necrosis) in one female and thymus atrophy in two females. It is noted that only reproductive organs were weighed, and only reproductive organs and gross lesions were examined histopathologically, so the information for assessment of target organ toxicity is limited.

Dose selection was based on a 14-d range-finding study (Anonymous, 2013a), in which 4 females per group were exposed to 0, 150 or 300 mg/kg bw/d of Omnirad 379. The top dose animals showed clinical signs (hunched posture, piloerection), body weight loss (2 out of 4 animals), reduced food consumption and increased liver weight.

Repeated dose studies with Omnirad 369

In a 14-d study (Anonymous, 1989a) Sprague-Dawley rats (5/sex/group) were administered Omnirad 369 via oral gavage at 0, 100, 300, 1000 and 3000 mg/kg bw/d. Effects at 300 mg/kg bw/d included increased liver weight in both sexes (absolute, by 24%/43% in m/f) and increased cholesterol in females. Mortality occurred in females at the two highest doses (1 and 3 animals at 1000 and 3000 mg/kg bw/d, respectively), other effects at these doses included reduced body weight and increased liver and adrenal weight. Histopathological examination was not performed.

In a subsequent 28-d study (Anonymous, 1989b) Sprague-Dawley rats (5/sex/group) were administered Omnirad 369 via oral gavage at 0, 10, 100 and 500 mg/kg bw/d. The weights of the liver, kidneys and adrenals were increased at the top dose without corresponding histopathological findings.

In another 28-d study (Anonymous, 2009) Wistar rats (5/sex/group) were administered Omnirad 369 via oral gavage at 0, 100 and 500/250 mg/kg bw/d. The top dose group started at 500 mg/kg bw/d, but the dose level had to be reduced to 250 mg/kg bw/d due to excessive toxicity; thus, 9 days at 500 mg/kg bw/d were followed by a 5-d recovery and a 28-d treatment at 250 mg/kg bw/d. Histopathological examination showed hyaline droplets in males, hepatocellular hypertrophy in both sexes and bone marrow atrophy in both sexes at the top dose. Absolute liver weight was increased by 13%/20% (m/f) and 25%/46% (m/f) at the mid- and top dose, respectively.

Conclusion

The main target organ effects below the (extrapolated) guidance values for classification in the available studies with Omnirad 379 were a modest increase in liver weight (without a histopathological correlate or clinical chemistry changes) and a moderate tubular hyaline change in males (not in females), probably representing accumulation of alpha$_{2u}$-globulin. RAC agrees that these effects are not of sufficient toxicological significance or severity to meet the classification criteria for STOT RE. Consequently, RAC agreed with the DS's proposal that **no classification for STOT RE is warranted**.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 31: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability			
BIOWIN V 4.10 (EPI Suite™)	Biowin 1= -0.26 (<0.5) Biowin 2= 0.00 (<0.5)	The substance is in the applicability domain of	Modelling, AT

Method	Results	Remarks	Reference
Substance: 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one	for Biowin 1 and 2, a fast degradation is defined as predicted probability >0.5 Biowin 3= 1.46 (<2.5) For Biowin 3, a fast ultimate degradation is defined as > 2.5 Biowin 4= 2.40 (<2.26 (-2.75)) A fast primary degradation is defined as > 2.5 Biowin 5= -0.22 (<0.5) Biowin 6= 0.0016 (<0.5) for Biowin 5 and 6, a fast degradation is defined as predicted probability >0.5 Biowin 7= -4.25 (<0.5) For Biowin 7 a fast degradation is defined as predicted probability > 0.5 Ready biodegradability prediction: **NO**	the used models, results are considered valid. Biowin 1 and 2: degradation is not fast, as values are below 0.5 Biowin 3 and 4: primary and ultimate degradations is not fast Biowin 5 and 6: not readily biodegradable based on linear and non-linear models Biowin 7: anaerobic degradation is not fast. Overall, substance is not readily biodegradable	
BIOWIN V 4.10 (EPI Suite™) Photolytic degradation product: 4-(4-morpholinyl)benz-aldehyde	Biowin 1= 0.39 (<0.5) Biowin 2= 0.86 (<0.5) Biowin 3= 2.54 (<2.5) Biowin 4= 3.47(<2.5) Biowin 5= 0.58 (<0.5) Biowin 6= 0.56 (<0.5) Biowin 7=-1.024 (<0.5) Based on Biowin 3 and 6 the substance does not biodegrade fast. Ready biodegradability prediction: **NO**	Biowin 3 result is borderline, as the value is higher than 2.5. Biowin 2 and 6 values are higher than the QSAR based screening criteria. The prediction for the overall degradability prediction is NO, which are based on Biowin 3 (≥2.75) and Biowin 5 results (≥0.5). Interpretation is difficult based on the obtained values. Metabolite is considered as borderline.	Modelling, AT
OECD Guideline 301B Ready biodegradability GLP 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113); purity 99.1%	-4.3 % of the initial dose was degraded within 9 days (based on CO_2) -5.3 % of the initial dose was degraded within 12 days (based on CO_2) -7.4 % of the initial dose was degraded within 28 days (based on CO_2)	Klimisch 1 AT: Klimisch 3 Toxicity control: The substance had an inhibitory effect on activated sludge microorganisms. Biodegradation rate in the toxicity control: 21% within 14 days. The test	Anonymous (2002b)

Method	Results	Remarks	Reference
Test item concentration: 20 mg/L, handeled in dark room with red light Sludge concentration: 30 mg/L Reference item: sodium benzoate; purity 99.6% Abiotic control: test item poisoned with mercury dichloride at 10 mg/L to inactivate microoragnisms Toxicity control: containing test item and reference item Positive control: containing reference item and inoculum Test duration: 28 days	Sodium benzoate used as positive control was readily biodegraded by an average of 80% within 14 days. Not readily biodegradable, due to the toxicity of the test item.	item can be assumed to be inhibitory. Activated slude was suitable, as indicated by the biodegradation of the reference item. Study was performed above the water solubility (~ 3 mg/L) of the test substance. No significant abiotic degradation was observed after 28 days.	
OECD Guideline 301C Ready biodegradability 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113); purity 99.1% Test concentration: 100 mg/L Sludge concentration: 30 mg/L Reference item: aniline Test duration: 28 days Test item was measured in triplicates	Not readily biodegradable under test conditions based on oxygen consumption	Klimisch 2 AT: Klimisch 4 For this study the full study report was not available. 61% of the reference substance aniline was degraded within 28 days (threshold 65% after 14 days in accordance to the OECD Guideline). Study was performed above the water solubility (~ 3 mg/L) of the test substance	Anonymous (2002c)
Hydrolysis			
OECD Guideline 111 Hydrolysis GLP	pH 4: hydrolytically stable, half-life > 1 year at 25 °C pH 7: hydrolytically stable, half-life > 1 year at 25 °C	Klimisch 1 Substance was hydrolytically stable under the applied conditions (pH 4, 7, 50°C, dark). Due to the low solubility in buffer	Anonymous (2002d)

Method	Results	Remarks	Reference
2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113); purity 99.1% Test temperature: 50°C; test performed in the dark; pH 4, 7, 9; test item dissolved in methanol and diluted with buffer	pH 9: test item was not detectable at any sample time	solution pH 9, the test item was not detectable at any sample time.	
Phototransformation			
OECD Guideline 316 Phototransformation of Chemicals in Water – Direct Photolysis GLP [Carbonyl-^{14}C] 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113); Radiochemical purity: 98.9%; Chemical purity: 97.5%	pH 4: Test concentration: 4.64 mg/L DT_{50} [hours] : 0.57 DT_{90} [hours]: 1.49 pH 7: Test concentration: 1.16 mg/L DT_{50} [hours] : 0.21 DT_{90} [hours]: 0.72 Seven major photolytic degradation products were detected which exceeded 10% of applied radioactivity (M1-M7), but only one was analytically identified.	Klimisch 1 Photolytic half-life in natural sun light is 26.6 minutes at pH 4 and 12.8 minutes at pH 7. A major degradation product M-3: 4-(4-morpholinyl)benz-aldehyde was detected (pH 7, light intensity 54.8-56.2 W/m^2) ≥ 10% of the applied dose. Degradation observed in the dark control samples, but no major degradation product formed.	Anonymous (2019a)
OECD Guideline 316 Phototransformation of Chemicals in Water – Direct Photolysis GLP [Carbonyl-14C] 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113); Radiochemical purity: 98.9%; Chemical purity: 97.5%	Different degradation products identified for M-2 (M-2a/b/c), M-3 (M-3a/b), M-4 (M-4a/b/c/d/e/f/g) and M-5 (M-5a/b). One degradation product identified for M-1, M-6 and M-7 and a structure was proposed. For M-3, two products were identified and a structure was proposed. Main degradation processes at pH 7: oxidation, reduction, desaturation, cleavage and hydrolysis (see Figure 2)	Klimisch 1 Due to limited or lacking data, molecular structure not proposed for all identified degradation products: M-2c, M-4e, M-4f, M-4g and M-5b	Anonymous (2019b) [follow up study to Anonymous, 2019a]

11.1.1 Ready biodegradability

Estimated data

The dossier submitter present QSAR calculations performed with BIOWIN v4.10 QSAR contained within EPI Suite™ version 4.10 (US-EPA, 2011) for EC 438-340-0 (Table 31). Biowin consists of 7 models. The substance is predicted to not biodegrade fast using linear (Biowin 1) and non-linear (Biowin 2) biodegradation models, as the values are below 0.5. The calculations are valid as the test substance is in the applicability domain of Biowin 1 and 2. EC 438-340-0 lies in the applicability domain of the models and is considered valid, as the substance is in the molecular range of the training set and many fragments of the substances are covered by the fragments of the training set (see Annex II). Ultimate biodegradation, the conversion from EC 438-340-0 to CO_2 (Biowin 3), is predicted not to occure fast. Initial steps, primary biodegradation are predicted to occure not fast, in days to weeks (Biowin 4). In Biowin 5 and 6, representing MITI testing, EC 438-340-0 was not considered to be readily biodegradable. Under anerobic conditions (Biowin 7), the test substnace is predicted not to quickly biodegrade. The overall prediction of the ready biodegradability is "no". The overall degradability prediction of the photolytic degradation product, 4-(4-morpholinyl)benzaldehyde is "no", but linear and non-linear models which score for rapid degradation are > 0.5, indicating fast degradation.

Experimental data

EC 438-340-0 was investigated for its ready biodegradability in a GLP study according to OECD Guideline 301B (Anonymous, 2002b). The study was performed with aerobic activated sludge (30 mg suspended solids/L) from a wastewater treatment plant treating domestic wastewater used to assess biodegradation in a culture medium with 20 mg test substance/L, which is above the water solubility of the test item (~3 mg/L (20°C)). Incubation was performed in 5-litre flasks at a temperature of 23-24°C in a dark room over 28 days. The pH value of the test item solution was 7.4. Produced CO_2 was captured in flasks containing 0.05M NaOH. Samples were taken on day 2, 5, 7, 9, 12, 14, 19, 23, 27 and 28 from the absorber flask nearest to the test flask. From the second absorber flask, samples were only taken on day 14 and 28. Additionally on day 29, samples were taken from both absorber flasks to determine residual CO_2 which was present in the suspension on day 28. A blank control sample, reference material (sodium benzoate) and a toxicity control were run in parallel for validity purposes.

As a results -7% biodegradation based on CO_2 evolution was observed for the substance after 28 days. The 10-day window failed. After 28 days, the abiotic control revealed -4.2% (based on CO_2). Sodium benzoate was readily biodegraded by an average of 80%. Therefore, the suitability of the activated sludge can be confirmed. EC 438-340-0 did not meet the pass level for biodegradation of 60% within 28 days. The substance had an inhibitory effect on activated sludge microorganisms. Biodegradation rate in the toxicity control revealed <25% starting from day 8 until the end of the test. According to OECD TG 301, the test item can be considered as inhibitory, if the test item and a reference compound are degraded less than 25% (based on $ThCO_2$). Therefore, the test is considered to not fulfill the validity criteria and was rated as Klimisch 3. The REACH registrant has assigned a reliability score of 1 (reliable without restriction) for this study. Over 28 days of test duration, it can be concluded that the test substance is not readily biodegradable, due to the toxicity of the test item.

Another ready biodegradability study was performed according to OECD Guideline 301C (modified MITI Test (I) under GLP) (Anonymous, 2002c). 100 mg 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one/L was incubated at 25°C with sludge collected from ten different places in Japan for four weeks. The test was conducted above the water solubility of the test substance. During incubation, the oxygen consumption was measured to determine the biodegradability. EC 438-340-0 was also analyzed quantitatively by HPLC after 28 days.

As a result over 28 days of incubation, the substance is considered not readily biodegradable. Nevertheless, it should be noted that only 61% of the reference substance (aniline) was degraded after 28 days. A full study report for a more detailed evaluation of the data was not available, therefore, the study was rated as Klimisch 4. The REACH registrant has allocated a reliability score of 2 (reliable with restrictions) to this study.

Conclusions on ready biodegradability

EC 438-340-0 is considered as not readily biodegradable, as the test item exhibited an inhibitory effect on the activated sluge microorganisms.

11.1.2 BOD_5/COD

Information of oxygen demand is not available.

11.1.3 Hydrolysis

For study summaries see Table 31.

Hydrolysis of 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113)) was tested in a GLP study according to OECD Guideline 111 in three buffer solutions of pH 4, 7 and 9 at a temperature of 50°C (Anonymous, 2002d). The temperature was kept constant with a water bath at ± 0.1 °C. As the test item is light sensitive, the hydrolysis experiment was performed in the dark. Duplicate samples were taken before incubation, after 2.4 hours and after 120 hours.

The results of pH 4 and 7 showed no significant degradation of the test substance (<10% degradation) at 50°C. At pH 9, the test substance was not detectable at any sampling time, indicating a low solubility in buffer solution pH 9. Based on this, it can be concluded that the substance is stable under environmentally relevant temperatures.

Conclusion hydrolysis

EC 438-340-0 is hydrolytically stable.

11.1.4 Other convincing scientific evidence

Other scientific evidence is not available.

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

Field investigations and monitoring data relevant for classification purposes are not available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Inherent and enhanced ready biodegradability tests are not available.

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

Water, water-sediment and soil degradation data (including simulation studies) are not available.

11.1.4.4 Photochemical degradation

For study summaries see Table 31.

As a result of a decision under REACH Article 41 a GLP study according to OECD Guideline 316 was performed by the REACH registrant. Radiolabelled 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005; CGI 113) was incubated in aqueous buffer solutions at pH 4 and pH 7 at an average temperature between 21.4 and 24.2°C for a maximum period of 14 days (Anonymous, 2019a). Final concentrations of the test substance were approximately 4.64 mg/L (pH 4) and 1.16 mg/L (pH 7). Wavelengths <290 nm were filtered out using a xenon arc lamp. Samples were taken at 12 time intervals within 14 days. To account loss of volume due to evaporation the weight of the test solution was also determined.

Results: At pH 4, the parent substance was not detectable anymore after 3 hours of incubation, while at pH 7 the parent substance was not detectable after 1 hour of incubation with simulated sunlight. Further seven major photolytic degradation products were determined (M-1 to M-7). Only product M-3 was identified as 4-(4-morpholinyl)benzaldehyde (CAS 1204-86-0) by co-chromatography against reference standard (see Figure 1). The degradability of M-3 based on Biowin results (EPI Suite v4.11) are difficult to interpret, as Biowin 3 results are borderline, and Biowin 2 and 6 values are higher than the QSAR based screening criteria (see Table 31) . Nevertheless, the prediction for the overall degradability prediction is NO, which are based on Biowin 3 (≥2.75) and Biowin 5 results (≥0.5). The other major degradation products were analyzed in an additional study (see Anonymous, 2019b). Degradation was also observed in the dark controls. 90.8% was recovered as parent at pH 4 and 88.7% was recovered as parent at pH 7 at day 14. No major degradation products were formed in the dark controls.

In conclusion, the photolytic half-life of EC 438-340-0 in natural sun light is 26.6 minutes at pH 4 and 12.8 minutes at pH 7.

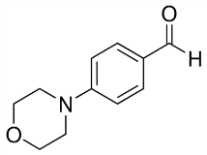


Figure 1: Degradation product M-3: 4-(4-morpholinyl)benzaldehyde

In an additional study by Anonymous (2019b), the detected seven major photolytic degradation products of EC 438-340-0 in water were analyzed using a LC-PDA-MS^n (Liquid chromatographic-Photodiode array-Mass spectrometer) method. It was observed that different degradation products were co-eluting at the retention time of M-2 (M-2a/b/c), M-3 (M-3a/b), M-4 (M-4a/b/c/d/e/f/g) and M-5 (M-5a/b), whilst one degradation product was identified for M-1, M-6 and M-7. On the basis of limited or lacking data, molecular structures were not proposed for all degradation products.

At pH 7 different relevant degradation processes were identified. Oxidation, reduction, hydrolysis, cleavage and desaturation are the main degradation processes of EC 438-340-0 in aquatic conditions at pH 7. In total ten different degradation products were formed at pH 7. The study author´s proposed following degradation pathway (Figure 2).

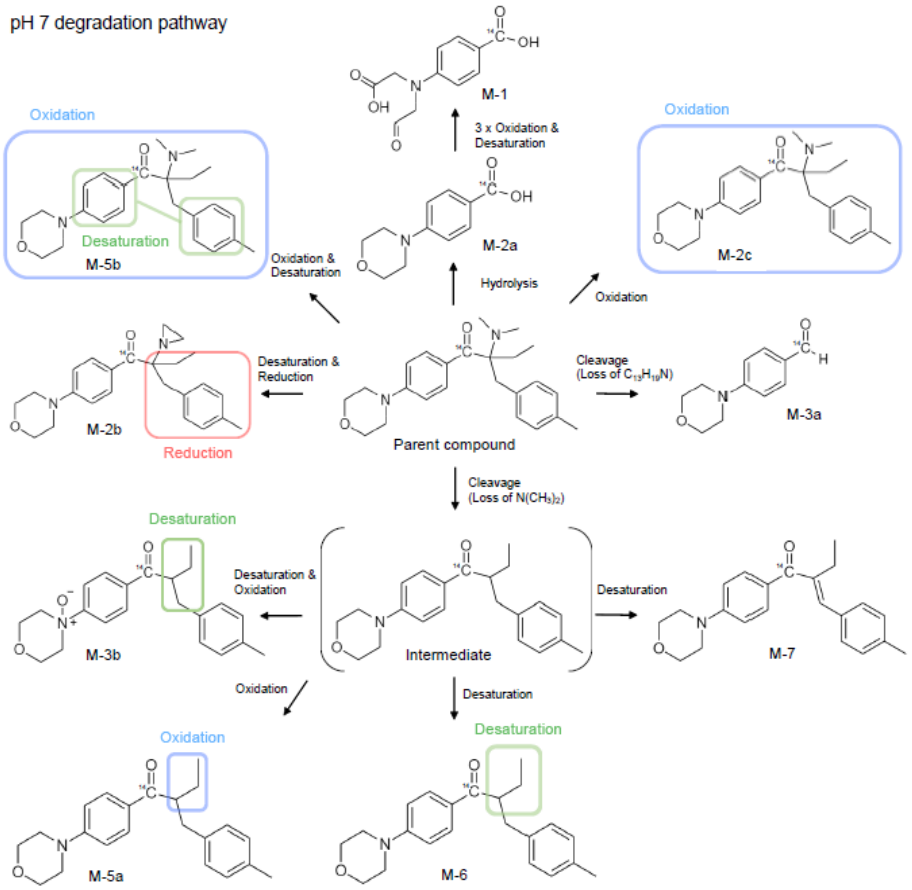


Figure 2: Degradation pathway of EC 438-340-0 at pH 7 proposed by the study Anonymous (2019b).

Conclusion photodegradation

Photodegradation of EC 438-340-0 in water has been observed at pH 4 and pH 7. Therefore, it is a relevant pathway in the degradation of the substance in aquatic conditions, it might be also relevant for soil surface.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Table 32: Summary of relevant information on environmental fate

Method	Results	Remarks	Reference
OECD Guideline 121 Estimation of the adsorption coefficient K_{oc} on Soil and Sewage sludge using High Performance Liquid	K_{oc} estimated by HPLC: log K_{oc} 3.5 (K_{oc} 3431) K_{oc} estimated by calculation: ≥ log K_{oc} 3.5 (≥ K_{oc} 3067)	Klimisch 2 It was not possible to determine the K_{oc} of the test substance with methanol/water as eluting solvent and with a CN-column as mentioned in the OECD guideline. An adjusted HPLC method with a gradient system was used and	Anonymous (2002e)

Method	Results	Remarks	Reference
Chromatography (HPLC) GLP 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one; purity 99.1% Test concentration: 125 mg/L Test item injected three times		additionally K_{oc} was estimated by using a calculation method.	

Adsorption

A GLP study according to OECD Guideline 121 "Estimation of the adsorption coefficient Koc on Soil and Sewage sludge using High Performance Liquid Chromatography (HPLC)" was conducted by Anonymous (2002e) to determine the adsorption coefficient (KOC). EC 438-340-0 was dissolved in methanol to get a test concentration of 125 mg/L. This solution was injected three times. The reference items were chosen in accordance to the OECD Guideline (isoproturon, triadimenol, linuron, fenthion, pyrazophos, DDT and Sodium nitrate for the determination of the dead time of the HPLC system). The reference solution was injected six times. The sodium nitrate solution was injected two times. As the test item was not elutable with methanol/water as mobile phase and with a CN-column as mentioned in the guideline, a HPLC method with a gradient system was used (Table 33). An analytical column packed with a commercially available phenyl solid phase was used in this study. Additionally the adsorption coefficient was estimated using a model calculation method based on a water solubility of ~3 mg/L using regression equations and relating the K_{OC} with the water solubility. Furthermore the molecular weight of the test substance, 380.53 g/mol, was used.

Table 33: Conditions of the HPLC System (Anonymous, 2002e).

Column: Bondapack Phenyl		
Eluent A: methanol and water (40:60; v/v); Eluent B: acetonitrile, ethanol and water (50:45:5; v/v/v)		
Time (minute)	gradient	
0	100% A	0% B
0-8	100% A	0% B
8-15	40% A	60% B
15-17	40% A	60% B
17-17.1	10% A	90% B
17.1-22	10% A	90% B
22-22.1	100% A	0% B
22.1-28	100% A	0% B
Temperature: 25°C, Injection volume: 20 µl		

The log K_{OC} was calculated using a regression curve and was found to be 3.5, which is equal to a K_{OC} value of 3431. Additionally the adsorption coefficient was also estimated to be ≥ 3067.

Conclusion on adsorption

EC 438-340-0 is considered as slightly mobile.

11.4 Bioaccumulation

Table 34: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD Guideline 117 Determination of the partition coefficient (n-octanol/water) GLP 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one; purity 99.1% Test concentration: 227 µg/mL (dissolved in methanol)	log Kow= 4.1 at 25°C, pH 8.1 Potential for bioaccumulation	Klimisch 1 AT: Klimisch 2 HPLC system with a gradient system was used instead of isocratic	Anonymous (2002f)
OECD Guideline 305 Bioconcentration study in Carp, *Cyprinus carpio* GLP 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-morpholin-4-yl)phenyl]butan-1-one; purity 99.1% High exposure level: 0.03 mg/L Low exposure level: 0.003 mg/L Flow-through system Uptake duration 28 days, Depuration duration 7 days Test temperature: 24.2 ± 0.7 °C	Mean BCF_{ss} at 0.03 mg/L: 755 L/Kg_{wwt} (whole fish) in *Cyprinus carpio* Mean BCF_{ss} at 0.003 mg/L: 684 L/Kg_{wwt} (whole fish) in *Cyprinus carpio* Potential for bioaccumulation	Klimisch 2 AT: Klimisch 2 No kinetic BCF (BCFk) available. BCF not normalised to a lipid content of 5% Tween 80 added as dispersant for both test concentrations The fish weight decreased during the study period in both test concentrations, indicating toxicity.	Anonymous (2002g)
BIOWIN v4.10 (EPI Suite™)	BCF: 61.2 L/Kg (regression based estimate)	Based on the measured logKow of 4.1 Result verified by AT	Registration dossier [accessed 04/2021]
Catalogic v5.11.17	BCF: 407.38 L/Kg		Registration dossier [accessed 04/2021]
T.E.S.T. v4.01	BCF: 101.39 L/Kg		Registration dossier [accessed 04/2021]
VEGA CAESAR v2.1.13	BCF: 10 L/Kg	Substance is out of the Applicability Domain of the model	Registration dossier [accessed 04/2021]

11.4.1 Estimated bioaccumulation

In the registration dossier results from different QSAR claculations were available. The calculated BCF values of different model calculations ranges from 10 L/Kg (VEGA) to 407.38 L/Kg (Catalogic). For the regression-based estimate of EPISuite the measured logKow of 4.1 was used. The result from the EPISuite calculation could be verified by AT. For the VEGA model the substance is out of the applicability domain of the model and the calculated value is therefore not reliable.

11.4.2 Measured partition coefficient and bioaccumulation test data

A study according to OECD Guideline 117 for the experimental partition coefficient n-octanol/water (log Kow) with the HPLC method was available (Anonymous, 2002f). The log Kow for EC 438-340-0 (=TK 11005 (CGI 113) was determined to be 4.1 at pH 8.1 and 25°C.

A study on the bioconcentration of EC 438-340-0 in *Cyprinus carpio* following OECD Guideline 305 was available (Anonymous, 2002g). The test fish were exposed to a concentration of 0.03 mg/L (high exposure level) and 0.003 mg/L (low exposure level) of the test substance in a flow-through system for 28 days. For both test concentrations Tween 80 was added as dispersant. An excretion test was conducted subsequent to the exposure test for seven days. The mean steady-state BCF was 755 L/Kg_{wwt} for the whole fish at the high exposure level and 684 L/Kg_{wwt} for the whole fish at the low exposure level. For both exposure levels the steady-state was at day 7. The mean residual rate of the test substance was 12 % in the high exposure level and 4 % in the low exposure level at day 7 of the excretion test. The validity criteria of the study were met. The weight of the fish were measured throughout the study duration. In the high exposure level the mean body weight of the fish decreased from 2.23 g to 1.87 g (n=4) during the study period. In the low exposure level the mean body weight of the fish decreased from 2.19 g to 1.64 g (n=4) during the study period. The lipid content of the fish were determined before and after the experiment. However, the BCF was not normalised to a lipid content of 5 % and a kinetic BCF (BCF_k) was not determined. No mortality was observed in the treatment groups and control group, but as the fish weight decreased during the study period toxicity cannot be ruled out.

Conclusion

The estimated BCF values were all below the cut-off value of 500. However, as the experimental log Kow is ≥ 4 and the experimental BCF for fish is ≥ 500 L/kg, it can be concluded that EC 438-340-0 has a significant potential for bioaccumulation in aquatic environments for classification purposes.

11.5 Acute aquatic hazard

Table 35: Summary of relevant information on acute aquatic toxicity. The test material was 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (EC 438-340-0) for all given studies/models

Method	Species	Results[1]	Remarks	Reference
OECD 203 (1992): 96h Fish Acute Toxicity Test GLP Static conditions EC 438-340-0, test concentration: 100 mg/L (nominal) Observed Endpoint: mortality, abnormalities Used Test concentration > water solubility Purity ≥ 99.1% Test temperature: 22.0 °C Dissolved oxygen: 8.0 – 8.8 mg/L pH: 7.8 – 7.9	*Brachydanio rerio*	96h-LC_{50}: > 0.13 mg/L mean measured based on geometric mean (100 mg/L nominal concentration)	Klimisch 1 AT: Klimisch 2	Anonymous (2002h)
QSAR estimation with ECOSAR v.11 EC 438-340-0 ECOSAR class not described	Fish	96h-LC_{50}: 4.161 mg/L	Klimisch 2 AT: Klimisch 3	Registration dossier (single submission, 2014) [accessed 04/2021]
QSAR estimation with ECOSAR version 2.0 using class “Aliphatic Amines” Input parameters: log Kow 4.1 and water solubility 2.8 mg/L. The substance EC 438-340-0 is in the applicability domain of the used model	Fish	96h-LC_{50}: 3.05 mg/L	Klimisch 2	Modelling, AT
OECD 202 (1984): 48h GLP Acute Immobilisation Test Static conditions EC 438-340-0, test concentration: 100 mg/L (nominal) Observed Endpoint: mobility Used Test concentration > water solubility Purity 99.1% Test temperature: 21.0°C Dissolved oxygen: 8.0 – 8.3 mg/L pH: 7.9	*Daphnia magna* Straus	48h-EC_{50}: not determined as the substance was neither measured at the beginning or end of the test (LOQ: 0.0643 mg/L)	Klimisch 1 AT: Klimisch 3	Anonymous (2002i)
QSAR estimation with ECOSAR v. 1.11 EC 438-340-0	Daphnid	48h-LC_{50}: 0.635 mg/L	Klimisch 2 AT: Klimisch 3	Registration dossier

ECOSAR class not described				(single submission, 2014) [accessed 04/2021]
QSAR estimation with ECOSAR version 2.0 using class "Aliphatic Amines" Input parameters: log Kow 4.1 and water solubility 2.8 mg/L The substance EC 438-340-0 is in the applicability domain of the used model	Daphnid	48h-LC_{50}: 0.46 mg/L	Klimisch 2	Modelling, AT
OECD 201 (1984): 72h GLP Algae growth inhibition test Static conditions EC 438-340-0, nominal test concentrations: 6.25, 12.5, 25, 50 and 100 mg/L Observed Endpoint: cell density, growth rate Used Test concentrations > water solubility Purity 99.1% Test temperature: 23.0 °C pH: 7.9 – 9.5	*Desmodesmus subspicatus*	72h-ErC_{50}: > 0.050 mg/L mean measured based on geometric mean (100 mg/L nominal concentration)	Klimisch 1 AT: Klimisch 2	Anonymous (2002j)
QSAR estimation with ECOSAR v. 1.11 EC 438-340-0 ECOSAR class not described	Green algae	96h-EC_{50}: 0.523 mg/L	Klimisch 2 AT: Klimisch 3	Registration dossier (single submission, 2014) [accessed 04/2021]
QSAR estimation with ECOSAR version 2.0 using class "Aliphatic Amines" Input parameters: log Kow 4.1 and water solubility 2.8 mg/L The substance EC 438-340-0 is in the applicability domain of the used model	Green algae	96h-EC_{50}: 0.24 mg/L	Klimisch 2	Modelling, AT

11.5.1 Acute (short-term) toxicity to fish

A limit test was performed according to OECD 203 (1992) following GLP (Anonymous, 2002h). The test organism zebra fish (*Danio rerio*) was exposed under static conditions to a nominal concentration of 100 mg/L of the test substance 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one. Auxiliary solvents or emulsifiers were not used to prepare the test media. The exposure solution was clear throughout the whole study duration. In parallel, a control without test medium was tested. The loading rate was lower than 1 g fish/L. Due to the light sensitive property of the test substance, the aquarium was wrapped

with black plastic foil. After 3, 24, 48, 72 and 96 hours the test organisms were observed for mortality and abnormalities. Analysis of the test concentrations was performed at the start and end of the test via HPLC. The analytical determined test substance concentration decreased from 0.28 mg/L at test start to 0.06 mg/L at the end of the test (0.13 mg/L mean measured based on geometric mean).

In a single REACH submission (2014) an LC_{50} value for fish is reported using ECOSAR. Nevertheless, no information on ECOSAR class is available.

A QSAR estimation using class "aliphatic amines" was performed by AT using ECOSAR version 2.0 with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L.

Results

The tested 96h-LC_{50} was at least higher than 0.13 mg/L based on geometric mean measured concentration.

In a single REACH submission (2014) Registrants estimated a value of 4.161 mg/L using ECOSAR v.1.11 (class not specified).

In an ECOSAR prediction performed by AT an LC_{50} value of 3.05 mg/L was gained for the class "aliphatic amines", which is slightly above the water solubility of 2.8 mg/L. Model and structure are applicable considering the type of structure and log Kow used. Therefore, the prediction is considered to be valid and reliable.

Conclusion

Both, experimental data (no toxicity observed up to the highest measured test concentration of 0.13 mg/L, mean measured based on geometric mean) and QSAR LC_{50} estimations (4.161 mg/L and 3.05 mg/L) show that EC 438-340-0 cannot be considered to be acutely toxic to fish.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

A limit test was performed according to OECD 202 (1984) following GLP (Anonymous, 2002i). Invertebrates (*Daphnia magna Straus*) were exposed under static conditions to a nominal concentration of 100 mg/L of the test substance EC 438-340-0.

Due to the low water solubility of the substance (acccording to a pre-test, not performed under GLP, the solubility limit of EC 438-340-0 in the test water was approximately 0.1 mg/L) a supersaturated dispersion with a loading rate of 100 mg/L was continuously stirred at room temperature in the dark over 3 hours. No auxiliary solvent or emulsifier was used. The supersaturated aqueous dispersion was filtered through a membrane filter (pore size 0.45 pm) just before the start of the test. The undiluted filtrate with the maximum concentration of dissolved substance was used as test medium. No observations were made concerning the test solution, as it was clear throughout the whole test duration. A control was tested in parallel.

For the treatment group and control group (without test medium) 20 daphnids were used, divided into two replicates of ten. The loading rate was lower than one daphnia per 2 mL test solution. For the analysis of the actual test item concentration samples were taken before test start, as also after 48 hours and analyzed via HPLC method. The measured concentration of the test item in the test solution was below the limit of quantification (LOQ) of 0.0643 mg/L at the start and at the end of the test. There was no limit of detection reported.

In a single REACH submission (2014) an LC_{50} value for aquatic invertebrates is reported using ECOSAR v.1.11. Nevertheless, no information on the used class is available.

A QSAR estimation was performed by AT using ECOSAR version 2.0 with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L (class "aliphatic amines").

Results

In the treatment group (nominal concentration of 100 mg/L), two organisms were immobile after 24 and 48 hours, but no EC_{50} could be established. As the substance could not be measured at the beginning or end of the study, the validity of this study is severly hampered. The test is not considered suitable for classification purposes.

In a single REACH submission (2014) Registrants reported an estimated LC_{50} value for aquatic invertebrates of 0.635 mg/L (ECOSAR v.1.11, class not specified).

A QSAR prediction performed by AT revealed an estimated LC_{50} value for daphnids of 0.46 mg/L for the class of "aliphatic amines" (ECOSAR v.2.0). Model and structure are applicable considering the type of structure and log Kow used. Therefore, the prediction is considered to be valid and reliable.

The relevance and reliability of the QSAR data are increased by the fact that the chronic values from QSAR estimations with fish and daphnia are in line with the outcome of recent experimental studies:

	Experimental chronic data	**QSAR estimation using ECOSAR version 2.0 (class "aliphatic amines") with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L**
Fish	32d-NOEC: 0.031 mg/L (average exposure concentration)	Chronic value: 0.08 mg/L
Daphnia	21d-NOEC for mortality, reproduction and growth: 0.064 mg/L (average exposure concentration) 21d-EC_{10} (reproduction): 0.065 mg/L (0.039-0.108 mg/L, 95% CI)	Chronic value: 0.05 mg/L

Conclusion

No acute toxicity value based on measured values could be derived from the experimental study. Therefore, the valid QSAR prediction estimating an LC_{50} value of 0.46 mg/L for daphnids is considered relevant. Therefore, EC 438-340-0 is assumed to comprise acute toxicity towards daphnids.

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

A static algae growth inhibition test by Anonymous (2002j) according to OECD 201 (1984) and GLP with the species *Desmodesmus subspicatus* was conducted with five nominal concentrations (100 mg/L, 50 mg/L, 25 mg/L, 12.5 mg/l and 6.25 mg/L corresponding to actually measured test concentrations of 165, 106, 55, 28, and 14 µg/L. Already the lowest nominal test concentration exceeds the water solubility of the test substance. The preparation of the test media was performed mostly in the dark to avoid photolytic degradation of the test item during handling. The substance was mixed into the test water by ultrasonic treatment for 15 minutes and by intense stirring for 3 hours at room temperature in the dark to dissolve a maximum concentrafion of the test item in the dispersion. For each test concentration, three replicates were used, whereas six replicates were used for the control (without test medium). Auxiliary solvents or emulsifiers were not used to prepare the test media. The test media of all concentrations was clear throughout the whole test duration. To determine the actual test item concentration samples were analyzed by HPLC. The actual test item concentration at 100 mg/L decreased from 165 µg/L at the beginning of the test to 15 µg/L at test end (50 µg/L mean measured based on geometric mean). For the other test concentrations the test concentration at the end was not determined. For the sampling test media without algae were used (in the beginning before algae were added, for the end the stability control was used).

In a single REACH submission (2014) an EC_{50} value for algae was reported using ECOSAR v.1.11. Nevertheless, no information on the used class is available.

A QSAR estimation using class "aliphatic amines" was performed by AT using ECOSAR version 2.0 with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L.

Results

During the whole test study the algae cell densities in the test mediums were equal or even higher than in the control culture. The 72h-ErC_{50} value was > 0.050 mg/L based on mean measured concentration (geometric

mean). As the acute toxicity value derived from the experimental study can only provide the information that the 72h-ErC_{50} value is > 0.050 mg/L based on mean measured concentration (geometric mean), this study can only be used up to this concentration.

In a single REACH submission (2014) Registrants estimated a value of 0.523 mg/L using ECOSAR v.1.11 (class not specified).

In an ECOSAR prediction performed by AT an EC_{50} value of 0.24 mg/L was gained for the class "aliphatic amines" (ECOSAR v.2.0 with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L). Model and structure are applicable considering the type of structure and log Kow used. Therefore, the prediction is considered to be valid and reliable.

The QSAR estimation performed by AT is considered reliable and the substance falls into the applicability domain of the model. The relevance and reliability of the QSAR data are increased by the fact that the chronic values from QSAR estimations with fish and daphnia are in line with the outcome of recent experimental studies:

	Experimental chronic data	**QSAR estimation using ECOSAR version 2.0 (class "aliphatic amines") with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L**
Fish	32d-NOEC: 0.031 mg/L (average exposure concentration)	Chronic value: 0.08 mg/L
Daphnia	21d-NOEC for mortality, reproduction and growth: 0.064 mg/L (average exposure concentration) 21d-EC_{10} (reproduction): 0.065 mg/L (0.039-0.108 mg/L, 95% CI)	Chronic value: 0.05 mg/L

Conclusion

As the acute toxicity value derived from the experimental study can only provide the information that the EC_{50} is above 0.05 mg/L, the valid QSAR prediction estimating an EC_{50} of 0.24 mg/L for algae is considered relevant. Therefore, EC 438-340-0 is assumed to comprise acute toxicity towards algae.

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

No date available.

11.6 Long-term aquatic hazard

Table 36: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
OECD 210 (2013): Fish, Early-life Stage Toxicity Test GLP Duration: 32 d Flow-through system	Fathead minnow (*Pimephales promelas*)	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK	32d-NOEC: 0.031 mg/L (average exposure concentration)	Klimisch 1 Key study	Anonymous (2019c)

Nominal test concentrations (µg/L): 50, 90, 160, 280, 500 'Used Test concentration < water solubility Purity ≥ 96.4% Test temperature: 23.7 – 25.3°C Dissolved oxygen: 4.6 – 9.5 mg/L pH: 7.1 – 7.7		11005 (CGI 113)			
QSAR estimation with ECOSAR version 2.0 using class "aliphatic amines" Input parameters: log Kow 4.1 and water solubility 2.8 mg/L The substance is in the applicability domain of the used model	Fish	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113)	ChV: 0.08 mg/L	Klimisch 2	Modelling, AT
Invertebrates					
OECD 211 (2012): Daphnia magna Reproduction Test Duration: 21 d GLP Flow-through system Nominal test concentrations (µg/L): 3.8, 12, 39, 125, 400 Used Test concentration < water solubility Purity ≥ 96.4% Test temperature: 18.0 – 22.0°C Dissolved oxygen: 2.9 – 9.7 mg/L pH: 7.5 – 8.2	*Daphnia magn*a	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113)	21d-NOEC for mortality, reproduction and growth: 0.064 mg/L (average exposure concentration) EC_{10} (reproduction): 0.065 mg/L (0.039-0.108 mg/L, 95% CI) EC_{10} (growth): 0.172 mg/L (0.118-0.250 mg/L, 95% CI)	Klimisch 1	Anonymous (2019d)
QSAR estimation with ECOSAR version 2.0 using class "aliphatic amines" Input parameters: log Kow 4.1 and water solubility 2.8 mg/L	Daphnid	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK	ChV: 0.05 mg/L	Klimisch 2	Modelling, AT

The substance is in the applicability domain of the used model		11005 (CGI 113)			
Algae					
OECD 201 (1984): 72h Algae growth inhibition test GLP Static conditions Nominal test concentrations: 6.25, 12.5, 25, 50 and 100 mg/L Observed Endpoint: cell density, growth rate Used Test concentrations > water solubility Purity 99.1% Test temperature: 23.0 °C pH: 7.9 – 9.5	*Desmodesmus subspicatus* (algae)	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113)	72h-NOEC: ≥ 0.050 mg/L mean measured based on geometric mean	Klimisch 2	Anonymous (2002j)
QSAR estimation with ECOSAR version 2.0 using class “aliphatic amines” Input parameters: log Kow 4.1 and water solubility 2.8 mg/L The substance is in the applicability domain of the used model	Green algae	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (=TK 11005 (CGI 113)	ChV: 0.09 mg/L	Klimisch 2	Modelling, AT

11.6.1 Chronic toxicity to fish

As a result of a decision under REACH Article 41 a chronic toxicity study according to the OECD test guideline 210 using *Pimephales promelas* was performed with the test substance 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one over a period of 32 days (Anonymous, 2019c).

In a range finding test including a blank and a solvent control (0.1 mL dimethylformamide (DMF)/L) the target nominal concentrations were 50, 90, 160, 280 and 500 µg/L. In the flow-through system the test item stock solutions were prepared using DMF (prepared three times a week with a factor 10,000 higher than target concentrations) and dosed via a computer-controlled system. The whole dosing system was checked twice daily on working days and once per day on weekends.

The study was performed with 80 fathead minnow embryos per test group, divided into four replicates of 20. The larvae and juvenile fish were fed ad libitum. On each day, the embryos and larvae were observed for survival. Effects on development, swimming behaviour and appearance were also recorded every day. At the test end all surviving fish were weighed and the individual length was measured. The actual test item concentrations were analysed via an Ultra Performance Liquid Chromatography (UPLC) system. Temperature, pH and the dissolved oxygen concentration were measured at the beginning, at weekly intervals and at the end of the test. Water hardness was measured at test start and test end in all test groups. In a blank medium, the

total organic carbon content was determined at the beginning of the test. At the end of the study, the surviving larvae were killed with 1.2% ethylene glycol monophenylether in water.

A QSAR estimation using class "aliphatic amines" was performed by AT using ECOSAR version 2.0 with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L.

Results

Table 37: Target, measured and average concentrations of the test item (Anonymous, 2019c).

Target concentration test item (µg/L)	Measured concentrations (µg/L)						Average exposure concentration (µg/L)
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 32	
50	25.2	31.0	35.4	31.5	32.8	32.7	31
90	18.0	20.7	56.5	52.2	58.3	51.2	43
160	52.0	78.4	94.5	87.7	93.5	84.6	82
280	77.4	140	154	130	151	129	130
500	127	245	323	224	262	224	234

The average measured concentrations were >20% below the target concentrations, although the analytical measurements revealed largely stable concentrations during the whole study.

The survival of the embryos was 99 % in the pooled controls (no statistically significant difference between the solvent and blank control). The embryonic survival was 98-100 % in the treatment groups up to and including the highest average measured concentration of 234 µg/L without any significant difference compared to the pooled controls (Table 38).

Table 38: Embryonic survival at the end of hatching (Anonymous, 2019c).

Average concentration test item (µg/L)	Total introduced (n)	Hatched (n)	Not Hatched (n)	Hatched (%)
Pooled control	160	158	2	99
Blank control	80	80	0	100
Solvent control	80	78	2	98
31	80	78	2	98
43	80	79	1	99
82	80	80	0	100
130	80	79	1	99
234	80	80	0	100

Table 39: Post-hatch survival at the end of exposure (Anonymous, 2019c).

Average concentration test item (µg/L)	Total hatched (n)	Survived (n)	Dead (n)	Post-hatch survival (%)
Pooled control	158	132	26	84
Blank control	80	71	9	89
Solvent control	78	61	17	78
31	78	68	10	87
43	79	76	3	96
82	80	68	12	85

130	79	57	22	72
234	80	58	22	73

The post-hatch larval survival was 84 % in the pooled controls (no statistically significant difference between the solvent and blank control) at the end of exposure. Post-hatch survival in the test concentrations was not statistically different from the pooled controls, although in the two highest concentrations a trend to lower post-hatch survival was observed.

In the treatment groups the post-hatch larval survival ranged between 72 and 96% at the end of exposure.

As there was no statistical significant difference between the control groups for body weight, the solvent and blank control were pooled (Table 40). At a mean measured concentration of 31 µg/L a not statistically significant reduction in body weight of 6% was observed, while fish exposed to the test item at mean measured concentrations between 43 µg/L and 234 µg/L showed a statistically significant reduction in body weight in the range of 21-28% ($p \leq 0.05$).

Table 40: Mean body weight and body weight reduction at the end of exposure (Anonymous, 2019c).

Average concentration test item (µg/L)	Mean body weight (mg)	Std. Dev.	n	Reduction (%)
Pooled control	76.65	7.59	8	-
Blank control	73.50	8.51	4	-
Solvent control	79.80	5.97	4	-
31	72.21	6.36	4	5.8
43	58.63	3.87	4	23.5*
82	60.30	3.66	4	21.3*
130	60.66	10.42	4	20.9*
234	54.95	5.90	4	28.3*

*statistically significant effect ($p \leq 0.05$)

Table 41: Mean body length and body length reduction at the end of exposure (Anonymous, 2019c).

Average concentration test item (µg/L)	Mean body length (mm)	Std. Dev.	n	Reduction (%)
Pooled control	21.01	0.47	8	-
Blank control	20.83		4	-
Solvent control	21.20		4	-
31	20.70	0.38	4	1.5
43	19.45	0.44	4	7.4*
82	19.45	0.17	4	7.4*
130	19.35	0.94	4	7.9*
234	18.97	0.54	4	9.7*

*statistically significant effect ($p \leq 0.05$)

As there was no statistical significant difference between the control groups for the body length, the solvent and blank control were pooled. In fish exposed to 31 µg/L a not statistically significant reduction in body length of 1.5% was observed. For fish exposed to an average concentration from 43 µg/L onwards a statistically significant reduction in body length in the range of 7-10% was observed in a concentration dependent manner ($p \leq 0.05$).

Based on these results, the NOEC for body weight and length was determined to be 31 µg/L. The validity criteria of the study were met, although there were slight deviations of the optimum level of dissolved oxygen (5.0 mg/L). At day 28 two measurements in the range of 4.6 and 4.9 mg/L were made at the average

concentration of 130 µg/L and one measurement of 4.6 mg/L was made at the average concentration of 234 µg/L. It is assumed that this slight deviations did not affect the toxicity of the test substance to the test organisms. At day 7, the dosing of the 43 µg/L concentration failed temporarily during the night. Samples were taken one hour after continuing the dosing and it could be shown that the measured concentrations at that time were already in agreement with measured concentrations at the test start.These deviations are considered of no relevance for the outcome of the study.

A QSAR prediction was performed by AT with an estimated ChV of 0.08 mg/L for the class of "aliphatic amines" (ECOSAR v. 2.0).

Conclusion

A 32d-NOEC of 0.031 mg/L based on average concentration was determined for EC 438-340-0 for growth based on statistically significant effects on body weight and length at 43 µg/L. No statistically significant effects on the hatching success or post-hatch larval survival up to and including the highest concentration of 234 µg/L were observed, although at the two highest concentrations not statistically significant reductions in post-hatch larval survival were observed.

The study is reliable and the NOEC can be used for classification purposes. The experimental data were underpinned by a QSAR estimation (ECOSAR version 2.0) revealing a chronic value of 0.08 mg/L for the class of "aliphatic amines". Model and structure are applicable considering the type of structure and log Kow used. Therefore, the prediction is considered to be valid and reliable.

11.6.2 Chronic toxicity to aquatic invertebrates

As a result of a decision under REACH Article 41 a chronic toxicity study to aquatic invertebrates was available (Anonymous, 2019d). *Daphnia magna* was exposed to EC 438-340-0 over a period of 21 days according to the OECD guideline 211 (2012).

On the basis of a range finding test a first final test with target test concentrations of 50, 90, 160, 280 and 500 µg/L and including a blank and a solvent control with 0.1 mL DMF/L was performed as a flow-through test. The corresponding actual average concentrations of the test item were 39, 74, 125, 222 and 349 µg/L. These analytically determined average measured concentrations were below the target concentrations but stable during the whole study.

As statistically significant effects on reproduction and body length were observed already at the lowest test concentration of 39 µg/L. A final test 2 with target test concentrations of 3.8, 12, 39, 125 and 400 µg/L was performed as well in a flow-through setting. The corresponding analytically determined average concentrations were 2.4, 8.3, 28, 64, 187 µg/L. The measured concentrations were as well quite below target concentrations but stable during the whole test duration.

In the flow-through system the test item stock solutions in DMF (prepared three times a week with a factor 10,000 higher than the target concentrations) were dosed via computer-controlled system. The dosing system was checked twice on working days and once per day on weekends.

For each test concentration and control group 20 daphnids divided into four groups of five were used. The condition of the parental daphnids was recorded on each day. At the end of the study duration, the length of the parental daphnids was measured. The actual test item concentrations were determined using a UPLC system. Temperature, pH and oxygen concentration of the test media was checked once a week (additionally temperature continuously monitored in a temperature-control vessel).

A QSAR estimation using class "aliphatic amines" was performed by AT using ECOSAR version 2.0 with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L.

Results

Range Finding Test

A total of 70% of the parental daphnids exposed to the highest nominal concentration of 500 µg/L, corresponding to an actual concentration of 215.3 µg/L based on 3 measurements, died during the test period. No statistically significant mortality was observed at any of the lower concentrations and the solvent control.

The surviving parental daphnids in the highest test concentration were observed to be smaller when compared to the control from day 5 on and were less coloured from day 8 on. One aborted egg was observed in the solvent control on day 8 and three aborted eggs were observed at the lowest test concentration on day 10.

Table 42: Mortality and Reproductive Potency during the Range-Finding Test (Anonymous, 2019d).

Target conc. test item (µg/L)	Average conc. test item (µg/L)	Daphnia exp. (n)	Cumulative number of dead/immobile parental daphnids (number of new neonate daphnids)									
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Solvent Control		5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (9)	0 (72)	1 (0)	1 (229)
		5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (20)	0 (71)	0 (0)	0 (347)
5.0	4.48*	5	0 (0)	0 (0)	1 (0)	1 (0)	1 (0)	1 (0)	1 (59)	1 (0)	1 (0)	2 (221)
		5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (24)	0 (76)	0 (1)	0 (381)
50	28.6**	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (62)	0 (13)	0 (1)	0 (291)
		5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (78)	0 (5)	0 (0)	0 (292)
500	215.3**	5	0 (0)	0 (0)	1 (0)	1 (0)	1 (0)	1 (0)	3 (0)	4 (0)	4 (0)	4 (0)
		5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	1 (0)	1 (0)	3 (0)

* Based on two calculated values (3.8 and 3.9) and one measured value (5.74)
**Based on three measurements on day -1, 0 and 3

The presence of eggs in the brood pouch was recorded for the first time on day 5 for the solvent control and the two lower test concentrations. The first brood was observed on day 7. For the highest test concentration the presence of eggs in the brood pouch was recorded on Day 6. However, no first brood was recorded during the 10-day test period.

Samples taken from the mixing vessels at the start of the test showed that the concentrations were in agreement with target (89-109%). Samples taken from the test concentrations at Day -1 (to check the flow-through system), Day 0 and Day 3 showed that the actual test concentrations were generally below target but largely stable.

Final Test 1

Table 43: Target, measured and average concentrations final test 1 (Anonymous, 2019d).

Target concentration test item (µg/L)	Measured concentrations (µg/L)				Average exposure concentration (µg/L)
	Day 0	Day 7	Day 14	Day 21	
50	41.2	37.8	39.2	37.0	39
90	82.7	73.2	70.5	70.5	74
160	130	110	130	130	125
280	212	219	204	251	222
500	310	397	290	402	349

These analytically determined average measured concentrations were below the target concentrations but largely stable during the whole study.

Table 44: Mortality (immobility) of parental daphnids in final test 1 (Anonymous, 2019d).

Average concentration (µg/L)	Total daphnids introduced (n)	Mobile (n)	Immobile (n)	Immobility (%)
Pooled control	40	39	1	2.5

Blank control	20	19	1	5.0
Solvent control	20	20	0	0
39	20	19	1	5.0
74	20	16	4	20.0
125	20	20	0	0
222	20	14	6	30.0*
349	20	16	4	20.0*

*statistically significant effect ($p \leq 0.05$)

As there was no statistically significant difference for mortality the solvent and blank control were pooled. Only one out of the forty introduced parental daphnids were immobile at the end of study in the combined control groups. A statistically significant increase in immobility was observed at concentrations of 222 and 349 µg/L with a mortality of 30% and 20%.

Table 45: Reduction of cumulative offspring per introduced parent at day 21 in final test 1 (Anonymous, 2019d).

Average concentration (µg/L)	Cumulative offspring Mean	Std.Dev	n	Reduction (%)
Pooled control	247.7	24.04	8	-
Blank control	231.9	13.78	4	-
Solvent control	263.4	22.24	4	-
39	202.3	17.73	4	18.3*
74	164.3	23.31	4	33.7*
125	173.0	8.52	4	30.2*
222	69.3	12.91	4	72.0*
349	9.8	7.85	4	96.0*

*statistically significant decrease ($p \leq 0.05$)

The blank and solvent control were pooled for reproduction as statistical analysis indicated no significant difference between these control groups. A statistically significant decrease compared to the pooled control groups was observed for reproduction at all test concentrations.

From the second lowest test concentration onwards immobile offspring was seen: 24 at 74µg/l, 119 at 125 µg/L, 94 at 222 µg/L and 33 at 349 µg/L. Aborted egges were observed from the test concentration 125 µg/L onwards: 35 at 125 µg/L, 52 at 222 µg/L and 11 at 349 µg/L.

Table 46: Group mean body length and reduction of length in final test 1 (Anonymous, 2019d).

Average concentration (µg/L)	Mean body length (mm)	Std. Dev.	n	Reduction (%)
Blank control	4.39	0.118	4	-
Solvent control	4.60	0.052	4	-
39	4.37	0.085	4	5.0*
74	4.51	0.100	4	1.9*
125	4.46	0.035	4	3.1*
222	4.16	0.067	4	9.5*
349	3.89	0.078	4	15.3*

*statistically significant effect ($p \leq 0.05$)

Comparing the control groups for body length via statistical analysis indicated a significant difference between the solvent and blank control. The solvent control was therefore used for statistical analysis. A statistically significant reduction in body length was observed in all test concentrations compared to the solvent control.

The validity criteria for the final test 1 can be assumed to be met, although the concentration of the dissolved oxygen decreased in the last week to 1.3 - 2.3 mg/L and was therefore < 3 mg/L.

The dissolved oxygen concentration in the controls met the validity criteria but there was a large difference with 8.1 mg/L in the blank control and 3.0 mg/L in the solvent control. The temperature measured in the control groups and treatment groups was within the optimum range but was not kept constant within 2°C.

As no NOEC could be established for reproduction and body length in final test 1 another test (final test 2) was conducted.

Final Test 2

Table 47: Target, measured and average concentrations final test 2 (Anonymous, 2019d).

Target concentration test item (µg/L)	Measured concentrations (µg/L)				Average exposure concentration (µg/L)
	Day 0	Day 7	Day 14	Day 21	
3.8	2.69	2.78	1.8	2.48	2.4
12	9.22	7.83	7.02	9.12	8.3
39	33.3	24.9	28.5	26.9	28
125	61.2	63.6	75.7	55.5	64
400	218	177	153	202	187

These analytically determined average measured concentrations were below the target concentrations but stable during the whole study.

Table 48: Mortility (immobility) of parental daphnids in final test 2 (Anonymous, 2019d).

Average concentration (µg/L)	Total daphnids introduced (n)	Mobile (n)	Immobile (n)	Immobility (%)
Pooled control	40	36	4	10
Blank control	20	19	1	5
Solvent control	20	17	3	15
2.4	20	18	2	10
8.3	20	14	6	30
28	20	16	4	20
64	20	16	4	20
187	20	5	15	75*

*statistically significant effect ($p \leq 0.05$)

In the combined control groups (no statistically significant difference between solvent and blank control), mortality of the parental daphnids was 10%. Following an analysis of contrasts the selected Step-down Cochran-Armitage test was performed.

A statistically significant increase of mortality (75%) was only observed at the highest test concentration of 187 µg/L, although not statistically significant higher values for mortality were observed from 8.3 µg/L onwards.

Table 49: Reduction of cumulative offspring per introduced parent at day 21 in final test 2 (Anonymous, 2019d).

Average concentration (µg/L)	Cumulative Offspring Mean	Std. Dev.	Introduced parents (n)	Reduction (%)
Pooled control	183	30.79	4	-
Blank control	192.4	21.41	4	-
Solvent control	173.1	38.80	8	-
2.4	189	42.22	4	-3.2

8.3	135	50.96	4	26.3
28	151	13.62	4	17.2
64	153	15.30	4	16.3
187	8	9.48	4	95.5*

*statistically significant effect ($p \leq 0.05$)

As no statistical significant difference for reproduction was found solvent and blank control were pooled.

Normality check according to Shapiro-Wilk´s test was passed, but variance homogeneity check according to Lenvene´s test on variance homogeneity failed and a multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm was made to establish significance.

Only at the highest concentration of 187 µg/L a statistically significant reduction in reproduction was observed, although starting from the mean measured concentration of 8.3 µg/L to 64 µg/L not statistically significant reductions in reproduction from 26.3 to 16.3%, respectively, were determined.

The numbers of immobile offspring were 0 in the blank control, 1 in the solvent control, 2 at 2.4µg/L, 3 at 8.3 µg/L, 4 at 28µg/L, 2 at 64 µg/L and 7 at 187 µg/L. Aborted eggs were found at the test concentrations of 8.3µg/L (2), 28µg/L (39), 64µg/L (9) and 187 (23).

Table 49: Group mean body length and reduction of length at day 21 in final test 2 (Anonymous, 2019d).

Average concentration (µg/L)	Mean body length (mm)	Std. Dev.	n	Reduction (n)
Pooled control	4.38	0.156	8	-
Blank control	4.43	0.041	4	-
Solvent control	4.32	0.217	4	-
2.4	4.37	0.142	4	0.1
8.3	4.42	0.121	4	-1.0
28	4.39	0.079	4	-0.2
64	4.38	0.052	4	-0.1
187	3.82	0.264	4	12.7*

*statistically significant effect ($p \leq 0.05$)

As no statistically significant difference between solvent and blank control for group mean body length was seen, these controls were pooled. At the highest concentration of 187 µg/L a statistically significant reduction of group mean body length of nearly 13% was observed leading to a 21d-NOEC of 0.064 mg/L.

Although not statistically significant effects were observed for reproduction and mortality from 8.3 µg/L, these were only statistically significant at 187 µg/L. At this concentration also a statistically significant decrease in group mean body length was seen. Therefore, it can be concluded that the NOEC for mortality, reproduction and growth was 0.064 mg/L based on average exposure concentrations.

The point estimates from the 3-param. normal cumulative distribution function (CDF) revealed an EC10 (reproduction) value of 65 µg/L (39-108 µg/L, 95% CI), but analysis of fit for the 3-param. normal CDF showed a significant lack of fit.

The point estimates from the 3-param. normal CDF revealed an EC10 (growth) value of 172 µg/L (118-250 µg/L, 95%CI).

The validity criteria for this study are considered to be met, although the dissolved oxygen concentration was < 3 mg/L on day 19 for the treatment group with an average concentration of 64 µg/L and the temperature measured in the control and treatment groups were not kept constant within 2°C (18-22°C). Nevertheless, it is assumed that these deviations had no influence on the outcome of the study.

A QSAR prediction performey by AT revealed an estimated ChV of 0.05 mg/L for the class of “aliphatic amines” (ECOSAR v. 2.0).

Conclusion

A NOEC based on mortality, reproduction and growth could be established at a concentration of 0.064 mg/L based on average concentration in the final test 2, although not statistically significant effects were also observed at lower concentrations and in final test 1 at the lowest test concentration of 39 µg/L statistically significant effects on reproduction and growth were observed. The standard deviations were rather high in final test 2 compared to final test 1. In both tests the validity criteria were met: the mortality of the parent animals did not exceed 20% at the end of the test and the mean numbers of living offspring produced per parent animal surviving were > 60 at the end of the test;

The study is reliable and the 21d-NOEC of 0.064 mg/L (mortality, reproduction and growth) can be used for classification purposes. The EC_{10} for reproduction is 0.065 mg/L and the EC_{10} for growth is 0.172 mg/L. Since there is a significant amount of variance explained by the regression model for both EC_{10} values and a significant lack of fit for the EC_{10} value for reproduction the NOEC is preferred for classification purposes.

The experimental data were underpinned by a QSAR estimation (ECOSAR version 2.0) revealing a chronic value of 0.05 mg/L for the class of "aliphatic amines". Model and structure are applicable considering the type of structure and log Kow used. Therefore, the prediction is considered to be valid and reliable.

11.6.3 Chronic toxicity to algae or other aquatic plants

For test description see section 11.5.3 (Anonymous, 2002j). As no toxicity was observed in the 72 hour algae study up to and including the highest test concentration of 50 µg/L the 72h-NOEC was ≥ 0.05 mg/L based on geometric mean measured concentrations.

A QSAR estimation using was performed by AT using ECOSAR version 2.0 (class "aliphatic amines") with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L.This estimation revealed a chronic value of 0.09 mg/L for the class of "Aliphatic Amines" for green algae.

As the chronic toxicity value derived from the experimental study can only provide the information that the NOEC is above 0.05 mg/L, this study can only be used up to this concentration. The QSAR prediction estimates a chronic value of 0.09 mg/L which is above 0.05 mg/L. The QSAR estimation is considered reliable and the substance falls into the applicability domain of the model. The relevance and reliability of the QSAR data are increased by the fact that the chronic values from QSAR estimations with fish and daphnia are in line with the outcome of recent experimental studies:

	Experimental chronic data	**QSAR estimation using ECOSAR version 2.0 (class "aliphatic amines") with the following input parameters: log Kow of 4.1 and water solubility of 2.8 mg/L**
Fish	32d-NOEC: 0.031 mg/L (average exposure concentration)	Chronic value: 0.08 mg/L
Daphnia	21d-NOEC for mortality, reproduction and growth: 0.064 mg/L (average exposure concentration) 21d-EC_{10} (reproduction): 0.065 mg/L (0.039-0.108 mg/L, 95% CI)	Chronic value: 0.05 mg/L

Conclusion

As the chronic toxicity value derived from the experimental study can only provide the information that the NOEC is above 0.05 mg/L, the valid QSAR prediction estimating a chronic value of 0.09 mg/L for algae is considered relevant. Therefore, EC 438-340-0 is assumed to comprise chronic toxicity towards algae.

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

As there are acute data available on fish, invertebrates and algae, there is a need to assess the criteria given in Table 4.1.0 (a) of the CLP Regulation. The classification would, subsequently, be according to the most stringent outcome.

Acute (short-term) aquatic hazard classification categories for hazardous to the aquatic environment:

Category Acute 1: (Note 1)	
96 hr LC 50 (for fish)	≤ 1 mg/l and/or
48 hr EC 50 (for crustacea)	≤ 1 mg/l and/or
72 or 96 hr ErC 50 (for algae or other aquatic plants)	≤ 1 mg/l. (Note 2)

Note 1: When classifying substances as Acute Category 1 and/or Chronic Category 1 it is necessary at the same time to indicate the appropriate M-factor(s) (see Table 4.1.3, CLP regulation).

Note 2: Classification shall be based on the ErC50 [= EC50 (growth rate)]. In circumstances where the basis of the EC50 is not specified or no ErC50 is recorded, classification shall be based on the lowest EC50 available.

Assessing the criteria of Table 4.1.0 (a), leads to classification as Aquatic Acute 1, based on the lowest acute 96h-EC_{50} value of 0.24 mg/L, which is derived from a QSAR estimation for algae. This classification is supported by an 48h-LC_{50} value of 0.46 mg/L, which is derived from a QSAR estimation for daphnids.

The experimental data for algae and daphnids are not used for classification, as the concentrations at which no effects occurred, could not be verified (daphnids) or were at lower concentrations than the available valid QSAR estimation (algae).

According to Table 4.1.3 of the CLP Regulation an M-Factor of 1 is warranted.

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

Bioaccumulation potential

The experimental determined log Kow is 4.1 at pH 8.1 and 25 °C. The experimental BCF is 684 L/kg_{wwt} and 755 L/kg_{wwt} for the whole fish. The estimated BCF values in the registration dossier are in the range from 10 L/Kg (VEGA) to 407.38 L/Kg (Catalogic).

As the experimental log Kow is ≥ 4 and the experimental BCF for fish is ≥ 500 L/kg, the substance has a potential to bioaccumulate in aquatic environments for classification purposes.

Rapid degradability

According to 4.1.2.9.5. of Annex I of Regulation (EC) No 1272/2008 (CLP Regulation) substances are considered rapidly degradable in the environment if one of the following criteria holds true:

(a) if, in 28-day ready biodegradation studies, at least the following levels of degradation are achieved:

(i) tests based on dissolved organic carbon: 70 %;

(ii) tests based on oxygen depletion or carbon dioxide generation: 60 % of theoretical maximum.

or (b) if, in those cases where only BOD and COD data are available, when the ratio of BOD 5 /COD is ≥ 0,5; or (c) if other convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level > 70 % within a 28-day period.

EC 438-340-0 is not readily biodegradable based on a 28-day test for ready biodegradability (OECD 301B), as the test item was toxic towards activated sludge microorganisms (Anonymous, 2002b) and is rated as Klimisch 3.

QSAR calulations with Biowin, predicted that EC 438-340-0 is does not biodegrade fast according to Biowin 1 and 2 and is not considered to be readily biodegradable based on Biowin 5 and 6. Furthermore, the overall prediction of the ready biodegradability is no.

The available data on hydrolysis (Anonymous, 2002d) does not support a conclusion on rapid degradability, as the half-life is > 1 year.

Photochemical degradation showed a half-life at environmental relevant conditions of 12.8 minutes. In total, 7 photolytic degradation products have been identified. One major metabolite was identified and is considered to be borderline according to estimated Biowin estimates (EPI Suite v4.11). No further experimental information is available on the degradability and toxicity of the so far only proposed, but not identified photolytic degradation products.

Regulation (EC) No 1272/2008, Annex I 4.1.2.9.3. allows the usage of degradation data that are available (degradation half-lives) and these can be used in defining rapid degradation provided that ultimate biodegradation of the substance, i.e. full mineralisation, is achieved. Primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

Photolysis seem to play a role in the degradation of EC 438-340-0 in water (Anonymous, 2019a,b) with a photolytic half-life of 26.6 minutes at pH 4 and 12.8 minutes at pH 7. Further seven major photolytic degradation products were determined (M-1 to M-7). Only M-3 was identified, as 4-(4-morpholinyl)benzaldehyde (CAS 1204-86-0), but for all metabolites no further information on toxicity and degradability is available. The ultimate degradation prediction indicates low rapid degradability.

Based on all information available, EC 438-340-0 can be considered as not rapidly degradable.

Long-term aquatic hazard

As there are long-term (chronic) data available on fish, invertebrates and algae, and the substance is not rapidly degradable there is a need to assess the criteria given in Table 4.1.0(b)(i) of the CLP Regulation. The classification would, subsequently, be according to the most stringent outcome.

(i) Non-rapidly degradable substances (Note 3) for which there are adequate chronic toxicity data available

Category Chronic 1: (Note 1)	
Chronic NOEC or EC x (for fish)	≤ 0,1 mg/l and/or
Chronic NOEC or EC x (for crustacea)	≤ 0,1 mg/l and/or
Chronic NOEC or EC x (for algae or other aquatic plants)	≤ 0,1 mg/l.
Category Chronic 2:	
Chronic NOEC or EC x (for fish)	> 0,1 to ≤ 1 mg/l and/or
Chronic NOEC or EC x (for crustacea)	> 0,1 to ≤ 1 mg/l and/or
Chronic NOEC or EC x (for algae or other aquatic plants)	> 0,1 to ≤ 1 mg/l.

Assessing the criteria of Table 4.1.0(b)(i), leads to classification as Aquatic Chronic 1, based on the lowest chronic 32d-NOEC of 0.031 mg/L, which was derived for growth in fish. This classification is supported by a 21d-NOEC for daphnia of 0.064 mg/l for mortality, reproduction and growth, a chronic value of 0.05 mg/L derived from an QSAR estimation for daphnids as well as a chronic value of 0.09 mg/L derived from an QSAR estimation for algae.

According to table 4.1.3 of the CLP Regulation an M-Factor of 1 is warranted.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the available data it is proposed to classify EC 438-340-0 as Aquatic Acute 1; 400 (Very toxic to aquatic life) with an acute M-factor = 1 and Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects) with a chronic M-factor = 1.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Aquatic Acute 1; H400 (very toxic to aquatic life) with an M-factor of 1, based on the 96h-EC_{50} value of 0.24 mg/L for green alga (derived by QSAR calculations, ECOSAR version 2.0 using the class "Aliphatic Amines"), and Aquatic Chronic 1; H410 (very toxic to aquatic life with long lasting effects) with an M-factor of 1, based on the 32 d-NOEC of 0.031 mg/L for fish growth (Fathead minnow (*Pimephales promelas*)) and the substance not being rapidly degradable.

Degradation

Ready biodegradability

The DS considered the substance as not readily biodegradable based on theoretical (QSAR) estimation and experimental studies presented below.

QSAR calculation: The dossier submitter presented QSAR calculations for Omnirad 379 performed with EPI (Estimation Programs Interface) SuiteTM version 4.10 (US-EPA, 2011) program BIOWIN. The results obtained can be accepted as valid as the substance falls within the applicability domain of the model. The calculations predict that the substance does not biodegrade fast according to Biowin 1 and 2 and that is not considered to be readily biodegradable based on Biowin 5 and 6.

Experimental data: Biodegradability of the substance Omnirad 379 was investigated in a GLP study according to OECD TG 301B (Anonymous, 2002b). Results showed 7% biodegradation based on CO_2 evolution for the substance after 28 days. The substance had an inhibitory effect on activated sludge microorganisms because the biodegradation rate in the toxicity control revealed <25% starting from day 8 until the end of the test. The DS concluded that the substance was not readily biodegradable, but rated the experimental study as of reliability 3 (Klimisch score) based on the test item toxicity towards activated sludge microorganisms.

Another ready biodegradability study for substance Omnirad 379 was performed according to OECD TG 301C (modified MITI Test (I) under GLP). Results indicated that over 28 days

of incubation, the substance was considered not readily biodegradable. The DS concluded that substance Omnirad 379 was not readily biodegradable and rated the study as Klimisch 4 due to the non availability of the full study report.

BOD_5/COD

Information of oxygen demand is not available.

Hydrolysis

A GLP compliant hydrolysis study was carried out according to OECD TG 111 in three buffer solutions of pH 4, 7 and 9 at a temperature of 50°C. The results of pH 4 and 7 showed no significant degradation of the test substance (<10% degradation) at 50°C. Based on this, it was concluded that the substance was hydrolytically stable under the tested temperatures and conditions.

Photochemical degradation

Photochemical degradation of the radiolabelled substance was studied in a GLP study according to OECD TG 316. Seven major photolytic degradation products were determined (M-1 to M-7). Only product M-3 was identified as 4-(4-morpholinyl)benzaldehyde (CAS 1204-86-0) by co-chromatography against reference standard. Degradation was also observed in the dark controls. 90.8% was recovered as parent at pH 4 and 88.7% was recovered as parent at pH 7 at day 14. No major degradation products were formed in the dark controls. The other major degradation products were analyzed in an additional study (see Anonymous, 2019b). The detected seven major photolytic degradation products of Omnirad 379 in water were identified using a LC-PDA-MSn (Liquid chromatographic-Photodiode array-Mass spectrometer) method (Anonymous (2019b). Oxidation, reduction, hydrolysis, cleavage and desaturation were the main degradation processes of EC 438-340-0 in aquatic conditions at pH 7. The degradation pathway was proposed in the study.

Environmental fate and other relevant information

Adsorption

Adsorption coefficient (K_{OC}) was determined by a GLP study according to OECD TG 121 "Estimation of the adsorption coefficient Koc on Soil and Sewage sludge using High Performance Liquid Chromatography (HPLC)" (Anonymous (2002e). The Log Koc was calculated using a regression curve (Log k' vs. Log Koc) and was found to be 3.5 which was equal to a Koc value of 3431. In the study the reference items covered the range of Log Koc 1.86 to Log Koc 5.63. The adsorption coefficient was also estimated to be ≥ 3067. The DS concluded that the substance was slightly mobile.

Bioaccumulation

Experimentally determined partition coefficient

Partition coefficient, n-octanol/water (Log K_{ow}) was determined according to OECD TG 117, using the HPLC method (Anonymous, 2002f). The Log Kow was determined to be 4.1 at pH 8.1 and 25°C.

Experimentally determined BCF

Only one experimental bioaccumulation study, following OECD TG 305 was available and considered by the DS as valid. (Anonymous, 2002g). The test fish *Cyprinus carpio* were exposed to a concentration of 0.03 mg/L (high exposure level) and 0.003 mg/L (low exposure level) of the test substance in a flow-through system for 28 days. The mean steady-state BCF was 755 L/Kg_{wwt} for the whole fish at the high exposure level and 684 L/Kg_{wwt} for the whole fish at the low exposure level. For both exposure levels the steady-state was reached at day 7. The mean residual rate of the test substance was 12 % in the high exposure level and 4 % in the low exposure level at day 7 of the excretion test. The BCF was not normalised to a lipid content of 5 % and a kinetic BCF (BCF_k) was not determined. No mortality was observed in the treatment groups and control group, but as the fish weight decreased during the study period toxicity, it cannot be ruled out.

Results for BCF from four QSAR model calculations were also available:

BIOWIN v4.10 (EPI Suite™) (BCF: 61.2 L/Kg);

CataLogic v5.11.17 (BCF: 407.38 L/Kg);

T.E.S.T. v4.01 (BCF: 101.39 L/Kg);

VEGA CAESAR v2.1.13 (BCF: 10 L/Kg)

The DS concluded that, as the experimental Log Kow was above 4 and the experimental BCF for fish was above the cut-off value of 500 L/kg, the substance has a potential for bioaccumulation in aquatic environments.

Acute aquatic hazard

A summary of relevant information on acute aquatic toxicity is presented in the CLH report.

Acute (short-term) toxicity to fish

One study performed on zebrafish (*Danio rerio*) according to OECD TG 203 (1992) following GLP was available for acute toxicity to fish (Anonymous, 2002h). The test was conducted under static conditions, with the suspension of test substance at nominal concentration of 100 mg/L in the test media treated in ultrasound bath for 15 min, stirred for 3 h and filtered through a membrane filter. The undiluted filtrate was tested as the only test concentration (= 0.28 mg/L) at the test start. The exposure solution was clear throughout the whole study duration. The loading rate was lower than 1 g fish/L. After 3, 24, 48, 72 and 96 hours the test organisms were observed for mortality and abnormalities. Analysis of the test concentrations was performed at the start and end of the test via HPLC. The substance concentration decreased from 0.28 mg/L at test start to 0.06 mg/L at the end of the test (0.17 mg/L mean measured based on all measurements or 0.13 mg/L geometric mean). No mortality or other visible abnormalities were determined during the test period of 96 hours.

The REACH Registrant estimated a value of 4.161 mg/L using ECOSAR version 1.11 (class not specified) (2014). An LC_{50} value of 3.05 mg/L was calculated by the DS using ECOSAR for the class "aliphatic amines", which was slightly above the water solubility of 2.8 mg/L. The model was applicable considering the substance molecular structure and Log Kow used.

The DS considered the LC_{50} value of 3.05 mg/L as valid and reliable. As a result, the DS concluded that Omnirad 379 cannot be considered acutely toxic to fish, taking into account the experimentally observed lack of toxicity at highest test concentration (LC_{50} >0.13 mg/L, measured geometric mean) and QSAR LC_{50} estimations of 4.161 mg/L and 3.05 mg/L.

Acute (short-term) toxicity to aquatic invertebrates

Acute toxicity toward invertebrates (*Daphnia magna Straus*) was studied in a limit test performed according to OECD TG 202 (1984) following GLP (Anonymous, 2002i). Test concentrations were achieved by the same procedure already described for the fish acute toxicity study. For the treatment group and control group (without test medium) 20 daphnids were used, divided into two replicates of ten and loading rate was lower than one daphnia per 2 mL test solution, under static conditions. Actual substance concentrations samples were measured at the start of the test and after 48 hours by HPLC method. The measured concentration was below the limit of quantification (LOQ) of 0.0643 mg/L at the start and at the end of the test. Two organisms were immobile after 24 and 48 hours but EC_{50} could not be established as the substance could not be measured. DS considered the test as not suitable for classification purposes.

The REACH Registrants estimated a value of 0.365 mg/L for aquatic invertebrates using ECOSAR version 1.11 (class not specified) (2014).

An LC_{50} value of 0.46 mg/L for daphnids was calculated by the Dossier Submitter using ECOSAR for the class "aliphatic amines". The model was applicable considering the substance molecular structure and Log Kow used. The DS concluded that an acute toxicity EC_{50} value for the substance could not be derived experimentally and considered, instead, the estimated QSAR value of LC_{50} 0.46 mg/L as relevant and suitable for classification.

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

A static algae growth inhibition study on *Desmodesmus subspicatus* was conducted according to OECD TG 201 (1984) and GLP Anonymous (2002j). Five nominal concentrations (6.25 - 100 mg/L) were tested, corresponding to actually measured by HPLC test concentrations of 0.165, 0.106, 0.055, 0.028, and 0.014 mg/L. Test media was prepared as described for fish acute toxicity study and further diluted. The actual substance concentration decreased from 0.165 mg/L at the beginning of the test to 0.015 mg/L at the end of the test (0.050 mg/L mean measured based on geometric mean). No other test concentrations were determined at the end of the test. During the whole test duration, the algae cell densities in the test mediums were equal to or even higher than in the control culture. The 72h-E_rC_{50}: > 0.050 mg/L mean measured based on geometric mean was defined.

The REACH Registrants estimated a value of 0.523 mg/L using ECOSAR version 1.11 (class not specified) (2014).

An EC_{50} value of 0.24 mg/L was calculated by the Dossier Submitter by ECOSAR version 2.0 for the class "aliphatic amines". The model was applicable considering the substance molecular structure and Log Kow used. The DS considered the EC_{50} value of 0.24 mg/L as valid and reliable.

The DS concluded that the acute toxicity value derived from the experimental study can only provide the information that the EC_{50} is above 0.05 mg/L, whilst considering relevant

the valid QSAR prediction. As such, the DS concluded that the EC_{50} value of 0.24 mg/L for algae estimated by ECOSAR version 2.0 leads to the classification of the substance as **Aquatic Acute 1 with an M-factor of 1.**

Long-term aquatic hazard

A summary of relevant information on chronic aquatic toxicity is presented in CLH dossier. The chronic toxicity test on fish and aquatic invertebrates have been conducted in response to an ECHA decision pursuant to Article 41 of the REACH Regulation:

https://www.echa.europa.eu/documents/10162/6d5d9fb5-5575-2b50-4f54-b8e1c0fb26d6

Chronic toxicity to fish

A chronic toxicity of substance to *Pimephales promelas* was performed according to the OECD TG 210 over a period of 32 days (Anonymous, 2019c). The test was performed in the flow-through system, test nominal concentrations 0.0550, 0.090, 0.160, 0.280 and 0.500 mg/L were prepared using 0.1 mL dimethylformamide (DMF)/L and dosed via a computer-controlled system, flow through. The study was performed with 80 fathead minnow embryos per test group, divided into four replicates of 20. The larvae and juvenile fish were fed ad libitum. On each day, the embryos and larvae were observed for survival. Effects on development, swimming behaviour and appearance were also recorded every day. At the test end all surviving fish were weighed and the individual length was measured. The actual substance concentrations were measured by Ultra Performance Liquid Chromatography system.

All conditions during the test were controlled. The embryonic survival was 98-100 % in the treatment groups up to and including the highest average measured concentration of 0.234 mg/L without any significant difference compared to the pooled controls. The post-hatch larval survival was 84 % in the pooled controls (no statistically significant difference between the solvent and blank control) at the end of exposure. Post-hatch survival for all concentrations was not statistically different from the pooled controls, although in the two highest concentrations a trend to lower post-hatch survival was observed. The post-hatch larval survival ranged between 72 and 96% at the end of exposure. The fish exposed to the test item at mean measured concentrations between 0.043 mg/L and 0.234 mg/L showed a statistically significant reduction in body weight in the range of 21-28% ($p \leq 0.05$). Fish exposed to average concentration from 0.043 mg/L onwards resulted in statistically significant reduction of body length in the range of 7-10% in a concentration dependent manner ($p \leq 0.05$). A NOEC for body weight and length was determined to be 0.031 mg/L. DS concluded that the validity criteria of the study were met although some slight deviations were observed and considered the study valid and reliable.

According to the DS, this value was supported by a QSAR estimation using class "aliphatic amines" that was performed using ECOSAR version 2.0, using a Log Kow of 4.1 and water solubility of 2.8 mg/L that derived an estimated chronic value of 0.08 mg/L. The DS consider the prediction valid and reliable, taking into account the substance molecular structure and Log Kow used.

Chronic toxicity to aquatic invertebrates

The chronic toxicity study to aquatic invertebrate *Daphnia magna* was available and conducted according to OECD TG 211 (2012) (Anonymous, 2019d). Test substance concentrations and test conditions were defined in the frame of a range finding test, followed by two other tests. In the first test (test 1), organisms were exposed over a period of 21 days at a nominal substance concentration of 0.050, 0.090, 0.160, 0.280 and 0.500 mg/L including a blank and a solvent control with 0.1 mL DMF/L, dosed via a computer-controlled system. The corresponding actual average concentrations of the substance were 0.039, 0.074, 0.125, 0.222 and 0.349 mg/L, measured by UPLC system. Validity criteria of the test 1 were met. A statistically significant increase in immobility was observed at concentrations of 0.222 and 0.349 mg/L with a mortality of 30% and 20%. Statistically significant effects on reproduction and body length were observed already at the lowest test concentration of 0.039 mg/L. In this way, no NOEC for reproduction and length were found within the range of concentrations used in test 1.

That was the reason for a second test (test 2) with nominal substance concentrations of 0.0038, 0.012, 0.039, 0.125 and 0.400 mg/L to be performed, also in a flow-through setting. The corresponding measured average concentrations were 0.0024, 0.0083, 0.028, 0.064, 0.187 mg/L, stable during the whole test. A control group of 20 daphnids divided into four groups of five species were exposed. The condition of the parental daphnids was recorded on each day. At the end of the study duration, the length of the parental daphnids was measured. Test conditions were carefully controlled. DS concluded that the validity criteria of the test are met. Statistically significant reduction in reproduction and mortality was observed at the highest concentration of 187 µg/L. At the highest concentration of 187 µg/L a statistically significant reduction of group mean body length of nearly 13% was observed leading to a 21d-NOEC of 0.064 mg/L.

The DS concluded that a lowest NOEC value based on mortality, reproduction and growth could be established at a concentration of 0.064 mg/L based on average concentration in the final test 2 and used for substance classification. The point estimates from the 3-param. normal cumulative distribution function (CDF) revealed an EC_{10} (reproduction) value of 65 µg/L (39-108 µg/L, 95% CI).

Application of QSAR estimation using class "aliphatic amines" and input parameters: Log Kow of 4.1 and water solubility of 2.8 mg/L revealed chronic value of 0.05 mg/L. DS considered the prediction to be valid and reliable.

Chronic toxicity to algae or other aquatic plants

A 72 hour algae growth inhibition test was conducted with *Desmodesmus subspicatus* according to OECD TG 201. Supersaturated suspension of test substance (100 mg/L) was filtered and used as highest test concentration, further concentrations obtained after 1:2; 1:4; 1:8 and 1:16 dilutions. Static conditions. Measured concentrations at the test at the start are: 0.165, 0.106, 0.549, 0.0283 and 0.0142 mg/L. Highest test concentration declined at the test end to 0.015 mg/L due to substance photosensitivity. No toxicity was observed in the study up to the highest achievable test concentration of 0.05 mg/L; the 72h-NOEC was thus above or equal to 0.05 mg/L, based on geometric mean measured concentrations.

Application of QSAR estimation using class "aliphatic amines" and input parameters Log Kow of 4.1 and water solubility of 2.8 mg/L revealed chronic value of 0.09 mg/L for green

algae. The DS considered the prediction to be valid and reliable taking into account that the experimentally derived NOEC value was above or equal to 0.05 mg/L. In addition, QSAR estimations with fish and daphnia were in line with the outcome of the recently conducted experimental studies.

The overall DS conclusion was that, based on valid experimental chronic toxicity data for all three trophic levels, a 32d-NOEC of 0.031 mg/L for body weight and length of *Pimephales promelas* leads to the classification of the substance as **Aquatic Chronic 1 with an M-factor of 1.**

Comments received during consultation

One MS commented on the results from chronic toxicity studies to aquatic invertebrate *Daphnia magna* rising the question why higher value of 0.064 mg/L for endpoint mortality, reproduction and growth (test 2) was preferred instead of the reproduction endpoint from test 1 that showed significant signs of toxicity at lower concentrations (<0.039 mg/L). A second comment sought clarification on the statistical significance of marginal differences in the group mean body length and reduction of length in test 1. The correctness of the three lowest test concentrations (mean measured concentrations of 39, 74 and 125 µg/L) significantly reducing the growth parameters was also raised. Finally, the MS supported the proposed classification Aquatic Acute 1 (M-factor=1) and Aquatic Chronic 1 (M-factor=1).

DS considered test 1 of the chronic toxicity to aquatic invertebrates as valid and agreed that as the effects on the reproduction endpoint were above 10% but below 20%, the NOEC could be calculated as LOEC/2, resulting in a NOEC of 0.0195 mg/L. The DS also cited the original study report, where the reductions in mean body length were statistically significant ($p \leq 0.05$) at all concentrations including the three lowest concentrations (Williams Multiple Sequential t-test Procedure was performed).

A second National Authority (NA) proposed to present lipid normalised BCFs. Concerning the ecotoxicity data, the NA emphasized that acute toxicity studies were available for fish, invertebrates and alga, with the validity criteria being met for the acute toxicity studies for fish and alga and showing no toxicity. Although the exposure concentrations in the acute toxicity study for invertebrates were not measured (<LOQ), the results were considered valid, meaning that the experimental results showed that acute effects were not observed up to the limit of maximum achievable solubility. The NA was unclear for the necessity of QSAR application and their preference for the estimation of key endpoint for the aquatic acute hazard and also required information for the limit of detection if available and discussion on substance solubility at pH>7.

DS re-calculated and presented the lipid normalisation BCF_{SSL} 821 L/kg for the high exposure level and 743f L/kg or the low exposure level. Concerning the acute aquatic invertebrates study, the DS pointed out that only LOQ was provided in the study and all results at the beginning or at the end of the study were below this value. DS considered this study not suitable for classification. Therefore, QSAR data were used.

Omnirad 379 is a weak base and solubility could be influenced by solution pH>7. DS pointed out that quantification of the substance was successful in the algae test at pH 8.0 (beginning) and 9.3 to 9.5 (end of the test) and substance was successfully measured each

of four days of the test so pKa value of 6.22 should not change substance water solubility in the aquatic invertebrate test.

Assessment and comparison with the classification criteria

Comparison with the CLP criteria

Parameter	CLP criteria	Results	Conclusion
Ready biodegradation	< 70% for 28 days	exhibited an inhibitory effect on the activated sludge microorganisms.	**Not rapidly degradable**
Bioaccumulation	Log K_{ow} ≥ 4 BCF ≥ 500 L/Kg	Log K_{ow} > 4 BCF > 500 L/Kg	**Potential for bioaccumulation**
Acute toxicity	EC_{50}	EC_{50} of 0.24 mg/L for algae estimated by ECOSAR version 2.0 for the class "aliphatic amines". supported	**Aquatic Acute 1; H400, M = 1**
Chronic toxicity	NOEC	32d-NOEC of 0.031 mg/L for body weight and length of *Pimephales promelas*	**Aquatic Chronic 1; H410, M = 1**

Rapid degradability

Experiments for rapid degradability showed that the test item was not readily degradable, with QSAR calculations confirming the experimental results, with the available data on hydrolysis (Anonymous, 2002d) indicating that the half-life was above one year. The substance was photo chemically unstable and primary biodegradation is not sufficient for the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment. RAC agrees with the DS conclusion that the substance is not rapidly degradable.

Bioaccumulation potential

Valid experimental values are available for Kow (4.1 at pH 8.1 and 25 °C) and bioaccumulation (BCF for fish ≥ 500 L/kg), leading to the RAC and DS conclusion that the substance has a potential to bioaccumulate in the aquatic environment.

Acute aquatic hazard

RAC agrees with the DS that experimental data for acute toxicity for fish, invertebrates and algae need to be supplemented by reliable QSAR calculations as, in the RAC opinion, reliable experimental data for aquatic invertebrates are not available. Acute toxicity tests were performed for fish, invertebrates and alga. The test substance solution for these tests was prepared according to a well-defined, identical procedure, namely filtration of 100 mg/L supersaturated solution of test substance (obtained after 15 min ultrasound treatment and 3 h stirring). Experimental data for fish showed no acute toxicity at highest measured test concentration of 0.28 mg/L, obtained after filtration of test substance suspension. Toxicity studies for alga were conducted with highest test substance concentration of 0.165 mg/L and, again, no toxicity was found.

Unexpectedly, using the same procedure for aquatic invertebrates, the highest measured test substance concentration was below the limit of quantification of the analytical method (0.0643 mg/L) at the start and at the end of the test medium renewal periods. RAC agrees with the DS that these experimental results are not valid and reliable for classification.

RAC additionally assessed the applicability and reliability of results obtained by ECOSAR version 2.0. The model classified the substance as aliphatic amine and ECOSAR models have been proven to show good accuracy predicting aliphatic amines and have a sufficient number of data points and statistical measures. They are considered, thus, scientifically valid. The prediction is within the applicability domain of the model as defined by model developers in terms of physicochemical properties (based on Log Kow and MW values) and structural domain (the model is specific for aliphatic amines, and the substance is an aliphatic amine). The aliphatic amine models have an $r^2 > 0.75$, which indicates good accuracy when predicting aliphatic amines in the training set.

Furthermore, the input value for Log Kow (=4.1,) is based on a reliable experimental data and lies within the power part of the range of available LogKow values in the model for this substance (predicted Log Kow by ECOSAR = 5.05, one experimental LogKow measurement of 5.73, but study details not accessible). This provides an indication that the substance toxicity may actually be somewhat underestimated by the model. Using as input a higher Log Kow may also lead to an acute classification also based on fish, but this has not been endorsed by the RAC, in the presence of valid experimental short-term toxicity study results for fish.

In conclusion, the available aquatic short-term database (valid experimental study results for fish and algae, supplemented by a valid QSAR-derived effects value of 0.46 mg/L for aquatic invertebrates) warrants a classification as **Aquatic Acute 1 with an M-factor of 1.**

Long-term aquatic hazard

Experimental, valid for classification, chronic toxicity data are available for fish, invertebrates and algae. RAC agrees with the commenting MS and considers the experimental results for *Daphnia magna* from both tests 1 and 2 as valid. When considering the available information from the tests, RAC proposes to use an aquatic invertebrate effects value based on the EC_{10} on reproduction (= 65 µg/L, 39-108 µg/L, 95% CI), in preference of a NOEC value for the same effects endpoint. Overall, considering that the substance is not rapidly degradable and based on the lowest chronic endpoint 32 d-NOEC of **0.031 mg/L** for *Pimephales promelas* body weight and length, RAC supports the DS and proposes to classify the substance in the category **Aquatic Chronic 1 with an M-factor of 1**.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not relevant.

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ANNEX I - STRUCTURAL ANALOGUE READ-ACROSS JUSTIFICATION FOR THE ENDPOINTS TOXICITY TO REPRODUCTION AND REPEATED DOSE TOXICITY

I – 1. Hypothesis for the analogue approach

In the following section the read-across has been described according to the Read-Across Guidance (ECHA, 2017) as well as ECHA guidance R.6 (2008).

In the present CLH-Dossier read-across using 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone (EC 404-360-3) as source substances has been applied to the endpoint toxicity to reproduction to support the available data and resulting classification of the target substance. Basis for the analogue approach is the high structural similarity and common toxicity.

2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone (EC 404-360-3) is used as source substance for read-across to the target substance 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (EC 438-340-0). Both substances (source and target substance) belong to the group of alkylaminoacetophenones and they differ only in one methyl group substitution (see Table 51).

In a compliance check according REACH it has also been concluded that the substances are structural analogues with high structural similarity and common toxicity is expected.

The table below gives an overview on relevant endpoints and available studies for the source and the target substance.

Table 50: Endpoints for which read-across applies and available studies for both substances.

	Scource substance **2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone** EC 404-360-3, CAS 119313-12-1 **Study type and reference**	**Target substance** **2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one** EC 438-340-0, CAS 119344-86-4 **Study type and reference**
Source	CLH-Dossier (BASF, 2015[1])	ECHA dissemination site and original study reports
Reproductive toxicity	OECD Guideline 415 (One-Generation Reproduction Toxicity Study) (Anonymous, 2011)	OECD 421 (Reproduction / Developmental Toxicity Screening Test) (Anonymous, 2013b)
	28-day dose-range finding study with additional investigations on fertility (Anonymous, 2009)	

[1] https://echa.europa.eu/documents/10162/82edd904-8cb3-c4aa-cc14-e1ce8c5813c0

	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) with macroscopic investigation of reproductive organs (Anonymous, 1989b)	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) (Anonymous, 2002)
	14-day dose-range finding study with determination of gonad weights (Anonymous, 1989a)	14-day dose-range finding study Anonymous, 2013a)

Reliability and adequacy of the source studies used for read-across

According to the ECHA (2008) Guidance on QSARs and grouping of chemicals, the used data for read-across needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy and reliability.

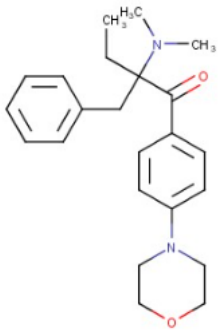
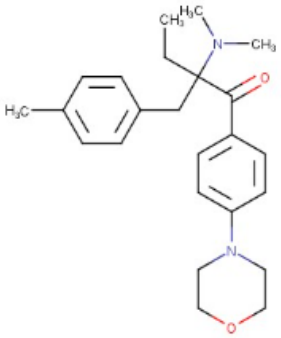
The studies for the source substance have been evaluated and discussed in the CLH-Dossier (BASF, 2015) and discussed at RAC (Sept 2016). The One-Generation Reproduction Toxicity study (Anonymous, 2011) and the Repeated-Dose 28 day Oral Toxicity Study (Anonymous, 1989b) have been rated as reliable without restrictions (Klimisch 1). Two dose-range finding studies (Anonymous 1989a, Anonymous, 2009) have been rated as reliable with restrictions (Klimisch 2).

I – 2. Identity and characterisation of the source substance and target substance

The identity of the source and target substances is compiled in the following table:

Table 51: Substance identities (ECHA dissemination site, 2020).

	Source substance	Target substance
Public name:	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one
EC number:	404-360-3	438-340-0
CAS number:	119313-12-1	119344-86-4
Molecular formula:	C23H30N2O2	C24H32N2O2
Molecular weight range [g/mol]:	366.5	380.5
Synonyms:	1-Butanone, 2-(dimethylamino)-1-[4-(4-morpholinyl)phenyl]-2-(phenylmethyl) Omnirad 369 Chivacure 169 IHT-PI 910	1-Butanone, 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(4-morpholinyl)phenyl]- Omnirad 379 JRCure 379

Chemical structure **(source:** European Chemicals Agency, http://echa.europa.eu/)		
Purity	98 – 99.9 % as a racemate[2]	racemate (info on purity confidential)
Harmonized classification	Repr. 1B, H360D Aquatic Acute 1, H400 Aquatic Chronic 1, H410	-

I – 3. Purity and Impuritites

For 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone the purity is documented in the CLH report (BASF, 2015) with a concentration range of 98 – 99.9 %. The substance is a racemate. One impurity is documented but is not relevant for classification.

For the target substance the purity is confidential and the substance is also a racemate. No impurities relevant for classification are documented.

I – 4. Analogue approach justification

Phys-chem properties

The source and the target substance belong to the group of alkylaminoacetophenones, differing only in a methyl group on the aromatic ring. This results in MWs of 366.5 and 380.5 for the source and the target substance, respectively. Both substances have a low water solubility and are lipophilic with partition coefficients (log Kow) of 2.91 and 4.1 (25°C).

For further information see Table 52.

Toxicity

Based on the available data the acute toxicity of both substances is low. Both substances have no irritating or sensitizing properties (see Table 53).

A subacute toxicity study with the target substance EC 438-340-0 shows that the main target organs after chronic exposure are the male genital system (testes, epididymides), the hematopoetic system and the liver in both sexes. General signs of toxicity are reduced bodyweight and reduced food consumption at high doses. Effect on the genital system at 450 mg/kg bw are described as test item related changes with organ weights of testes (-49%, $p<0.01$) and epididymides (-34%, $p<0.01$) in rats. Macroscopic observations show small testes and microscopic examination demonstrated that testes exhibited moderately to markedly reduced spermatogenesis and occurrence of spermatic giant cells, not reversible during recovery.

A reproductive screening study with EC 438-340-0 is available showing no toxicologically relevant effects on reproductive parameters up to 200 mg/kg bw/day. Absolute and relative weight of the epididymides was

[2] https://echa.europa.eu/documents/10162/82edd904-8cb3-c4aa-cc14-e1ce8c5813c0

statistically significantly reduced at 200 mg/kg bw/day (-17%) and microscopic findings in testes and epididymides show intraluminal cell debris of the epididymides, oligospermia of the epididymides and germ cell exfoliating into the lumen of seminiferous tubules of the testes. At 200 mg/kg bw/day developmental toxicity was noted consisting of an increased number of dead pups at first litter check and postnatal loss, and a decreased number of living pups at first litter check, viability index and body weights of the pups

Repeated dose studies with the source substance EC 404-360-3 (Anonymous, 1989b) show effects predominantly on liver weight, body weight and food consumption at concentrations up to 500 mg/kg bw. In a one-generation study (Anonymous, 2011) changes in the weights of male reproductive organs (without histopathological and functional changes) as well as an increase in both pre- and postnatal pup mortality as well as a decrease in pup weight were observed resulting in a harmonized classification as Repr 1B, H360D (RAC opinion, 2016) .

Both substances show similar toxicity at similar doses. Target organs of common systemic toxicity are liver, kidney and adrenals as well as the male reproductive system. Effects on developmental toxicity are characterized by reduced viability indexes, stillborn pups, postnatal mortality and reduced pup body weights.

I – 5. Data Matrix of selected physicochemical and toxicological information

I -5.1 Physicochemcial data

Table 52: Phys-chem properties of source and target substance (ECHA dissemination site, accessed Feb 2020)

Read-across	Source substance	Target substance
Chemical name	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one
State of the substance at 20°C and 101.3 kPa	solid	solid
Melting point	113.2°C	68°C
Boiling point	- decomposition at >275°C	- decomposition at 270°C
Relative density	1.21	1.16
Vapour pressure	0 Pa (25°C)	0 Pa (25°C)
Partition coefficient n-octanol/water	2.91 (25°C, pH 7)	4.1 (25°C, pH 8.1)
Water solubility	5.9 mg/L (20°C)	1.9 mg/L (20°C)

I -5.2 Toxicological data

Information on toxicological endpoints based on ECHA dissemination site, original study reports and the CLH report for EC 404-360-3 (BASF, 2015) is compiled in Table 53.

Table 53: Toxicological data of source and target substance

Read-across	Source substance	Target substance
Chemical name	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one
Acute Tox Oral	LD_{50} > 5000 mg/kg bw	LD_{50} > 2000 mg/kg bw

Acute Tox Dermal	LD_{50} > 2000 mg/kg bw	LD_{50} > 2000 mg/kg bw
Acute Tox Inhalation	No data	No data
Skin irritation	No irritating	No irritating
Eye irritation	No irritating	No irritating
Sensitization	Not sensitizing	Not sensitizing
Subacute toxicity studies (oral)	**28-day study** (0, 10, 50, 500 mg/kg bw): - NOEL = 10 mg/kg bw - Slight, reversible increase in adrenal weights (f) at 50 mg/kg bw (absolute +23%) - Effects on haematology, biochemistry and urinary parameters at 500 mg/kg bw - Slight effects on kidney weights, reversible within 14 days at 500 mg/kg bw - Increased liver weight (+41% m, +87% f) at 500 mg/kg bw, no histopathological abnormalities - Alopecia in all f and 1/5 m at 500 mg/kg bw - No macroscopic abnormalities for reproductive organs **14-day range finding study** (0, 100, 300, 1000, 3000 mg/kg bw): - NOEL = 100 mg/kg bw - Reduced bw and food consumption at 300 mg/kg bw - Increased liver and adrenal weights at ≥300 mg/kg bw - Severe bw loss and mortality at 1000 and 3000 mg/kg bw - No changes in relative gonad weights in females **28-day range finding study** (0, 100, 250/500 mg/kg bw): - NOAEL = 100 mg/kg bw - Effects on hematology/clinical chem., discolouration and increased kidney weights at highest dose	**28-day study** (0, 15, 50, 150, 450 mg/kg bw): - NOAEL = 50 mg/kg bw - Reduces testis size, weight of testes and epididymides and reduced spermatogenesis (associated with spermic giant cells and tubular atrophy) at 450 mg/kg bw - Hyaline changes in kidneys of m at 150 and 450 mg/kg bw - Fatty atrophy in bone marrow (450 mg/kg bw) - Increased splenic extramedullary hematopoetic activity at 50 mg/kg bw and above in m - Hematology: test-item related effects in both sexes at 150 and 450 mg/kg bw/day; haemoglobin and haematocrit values decreased at 450 mg/kg bw - Increased liver weight in f at 50 mg/kg bw, inceased liver and kidney weights in m and f at 150 and 450 mg/kg bw - Slight centrilobular hepatocellular hypertrophy in f at 450 mg/kg bw - Reduced bw in m at 150 mg/kg bw and both at 450 mg/kg bw **14-day range finding study** (0, 150, 300 mg/kg bw): - NOAEL < 150 mg/kg bw - 150 mg/kg: slight bw loss in 2f, slighty reduced food consumption (d 5-10),

	- Liver histopathological changes (hypertrophy of hepatocytes), increased liver weights at highest dose (+25% m, +45% f) - No macroscopic findings in epididymides and reproductive organs (weights not determined) - Normal spermatogenesis, no effects on sperm motility	- increased liver weights in all f, increased weight of adrenal glands in 1f - 300 mg/kg bw: bw loss in 2f, reduced food consumption (d 1-10), enlarged liver in 4f, enlarged adrenal glands in 2f, increased liver weights in all f, increased weight of adrenal glands in 2f; hunched posture, piloerection, rales (0-3h after dosing),
Sexual function and Fertility (oral)	**OECD 415** (0, 30. 100, 300 mg/kg bw): - Changes in weight of male reproductive organs at 300 mg/kg bw (testes ↑, prostate ↓, seminal vesicle ↓) - No histopathological/ functional changes - No effects in female reproductive organs	**OECD 421** (0, 20, 60, 200 mg/kg bw) - NOAEL (systemic tox) = 20 mg/kg bw - NOAEL (fertility, m) = 60 mg/kg bw 200 mg/kg bw: - Reduced bw gain in m; bw loss in f week 1 of treatment, decreased food consumption in f - Lymphoid atrophy (moderate) of the thymus in 2/10 females - Decreased terminal body weights in m, reduced epididymides weight (-17%); intraluminal cell debris of the epididymides in 8/10 males (up to moderate degree), oligospermia of the epididymides in 4/10 males (up to slight degree) - Germ cell exfoliating into the lumen of seminiferous tubules of the testes (without degeneration) in 9/10 males (up to moderate degree) 60 mg/kg bw: - Decreased terminal body weights in m, reduced bw gain in m, slightly higher testis weights
Adverse effects on development (oral)	**OECD 415** (0, 30, 100, 300 mg/kg bw): - NOAEL (development) = 30 mg/kg bw	**OECD 421** (0, 20, 60, 200 mg/kg bw) - NOAEL (development) = 60 mg/kg bw

	- Signif. increased number of stillborn pups at at 100 and 300 mg/kg bw (dose-related) - Signif. increased postnatal mortality at 300 mg/kg bw, viability index 86% - Decrease in pup body weight (dose related, signif. at 300 mg/kg bw) - Maternal toxicity: increased liver weights at mid (114%) and high dose (144%), reduced bw/bw gain, reduced food consumption, increased adrenal weights (mid and high dose, stress adaptive)	Effects at 200 mg/kg bw: - Dead pups (20) at first litter check from 2/10 females - Signif. reduced viability index (87.9%) - Postnatal loss (-12.1%) in 4/10 litters - Reduced pup body weights - Parental toxicity at 200 mg/kg bw: Reduced bw gain in m; bw loss in f week 1 of treatment, decreased food consumption in f; Lymphoid atrophy (moderate) of the thymus in 2/10 females
Mutagenicity	Negative	Negative
Carcinogenicity	No data	No data

I – 6. Conclusion

An analogue read-across approach between 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone (source chemical) (EC 404-360-3) and 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(morpholin-4-yl)phenyl]butan-1-one (target chemical) (EC 438-340-0) has been applied based on similarities in structure and toxicity.

Reproductive toxicity

For the target chemical (EC 438-340-0) a screening study is available and read-across data (EC 404-360-3) was used to support the available data and conclusion on reproductive toxicity. Both substances show similar toxicity at similar doses.

The OECD 421 screening study carried out with EC 438-340-0 (target chemical) showed effects on the male genital system (reduced epididymides weight, microscopic findings in testes and epididymides) at 200 mg/kg bw. At this concentration also developmental toxicity was noted consisting of an increased number of dead pups at first litter check, postnatal loss, and decreased viability index and body weights of the pups. In a subacute toxicity study with EC 438-340-0 the male genital system (testes, epididymides) could also be identified as main target (reduced organ weights of testes and epididymides, reduced spermatogenesis).

In a one-generation study with the source substance EC 404-360-3 changes in the weights of male reproductive organs (without histopathological and functional changes) as well as an increase in both pre- and postnatal pup mortality as well as a decrease in pup weight were observed resulting in a harmonized classification as Repr 1B, H360D (RAC opinion, 2016) .

It can be summarized that target organs of common systemic toxicity of these similar substances are liver, kidney and adrenals as well as the male reproductive system. Effects on developmental toxicity are characterized by reduced viability indexes, stillborn pups, postnatal mortality and reduced pup body weights.

No information on a possible mode of action is available, neither for the source nor for the target substance.

Based on thorough analysis of all available information a read-across approach for the endpoint reproductive toxicity is considered appropriate.

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ANNEX I I- BIOWIN 1 AND 2: APPLICABILITY DOMAIN

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------------------------- BIOWIN v4.10 Results ----------------------------


   Biowin1 (Linear Model Prediction)    :  Does Not Biodegrade Fast
   Biowin2 (Non-Linear Model Prediction):  Does Not Biodegrade Fast
   Biowin3 (Ultimate Biodegradation Timeframe):  Recalcitrant
   Biowin4 (Primary  Biodegradation Timeframe):  Weeks-Months
   Biowin5 (MITI Linear Model Prediction)    :  Does Not Biodegrade Fast
   Biowin6 (MITI Non-Linear Model Prediction):  Does Not Biodegrade Fast
   Biowin7 (Anaerobic Model Prediction):  Does Not Biodegrade Fast
   Ready Biodegradability Prediction:  NO
```

TYPE	NUM	Biowin1 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Carbon with 4 single bonds & no hydrogens	-0.1839	-0.1839
Frag	1	Aliphatic ether [C-O-C]	-0.3474	-0.3474
Frag	1	Ketone [-C-C(=O)-C-]	0.0068	0.0068
Frag	2	Tertiary amine	-0.2053	-0.4105
Frag	2	Alkyl substituent on aromatic ring	0.0547	0.1093
MolWt	*	Molecular Weight Parameter		-0.1812
Const	*	Equation Constant		0.7475
RESULT		Biowin1 (Linear Biodeg Probability)		-0.2593

TYPE	NUM	Biowin2 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Carbon with 4 single bonds & no hydrogens	-1.7232	-1.7232
Frag	1	Aliphatic ether [C-O-C]	-3.4294	-3.4294
Frag	1	Ketone [-C-C(=O)-C-]	-0.4530	-0.4530
Frag	2	Tertiary amine	-2.2229	-4.4458
Frag	2	Alkyl substituent on aromatic ring	0.5771	1.1542
MolWt	*	Molecular Weight Parameter		-5.4036
RESULT		Biowin2 (Non-Linear Biodeg Probability)		0.0000

```
 A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
 A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast
```

Table 54: Identity of fragments in EC 438-340-0 and frequency of fragment in trainings

Fragment	Present in EC 430-340-0	Number of compounds in the 295 compound training set containing the fragment (Annex D, Biowin help)
Aliphatic ether [C-O-C]	1	11
Ketone [-C-C(=O)-C-]	1	12
Tertiary amine	2	10
Alkyl substituent on aromatic ring	2	36