Analysis of the most appropriate risk management option (RMOA)

Substance Name: 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran (HHCB)

EC Number: 214-946-9

CAS Number: 1222-05-5

Authority: FRANCE

Date: August 2019

Public version.

Cover Note

In the framework of the French National Strategy on Endocrine Disruptors in 2018, the French Competent Authority requested ANSES to evaluate the ED properties of HHCB and verify whether risk management measures should be necessary for this substance. The PBT potential of the substance has also been assessed.

Comments and additional relevant information are invited on this RMOA by 30 september 2019.

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1 IDENTITY OF THE SUBSTANCE

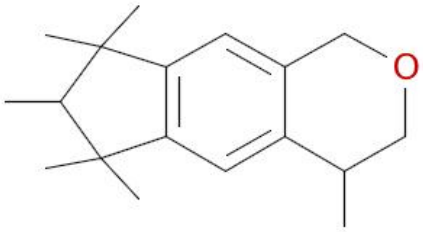
1.1 Other identifiers of the substance

Table: Other Substance identifiers

EC name (public):	HHCB
IUPAC name (public):	4,6,6,7,8,8-hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isochromene
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	C18H26O
Molecular weight or molecular weight range:	258.3984
Synonyms:	*Cyclopenta(g)-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-* 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran *Galaxolide*

Type of substance ☐ Mono-constituent ☒ Multi-constituent ☐ UVCB

Structural formula:



1.2 Similar substances/grouping possibilities

Not relevant in the frame of this RMOA.

2 OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Table: Completed or ongoing processes

RMOA		☐ Risk Management Option Analysis (RMOA) other than this RMOA
REACH Processes	Evaluation	☒ Compliance check, Final decision
		☐ Testing proposal
		☐ CoRAP and Substance Evaluation
	Authorisation	☐ Candidate List
		☐ Annex XIV
	Restri -ction	☐ Annex XVII[1]
Harmonised C&L		☐ Annex VI (CLP) (see section 3.1)
Processes under other EU legislation		☐ Plant Protection Products Regulation Regulation (EC) No 1107/2009
		☐ Biocidal Product Regulation Regulation (EU) 528/2012 and amendments
Previous legislation		☐ Dangerous substances Directive Directive 67/548/EEC (NONS)
		☒ Existing Substances Regulation Regulation 793/93/EEC (RAR/RRS)

[1] Please specify the relevant entry.

(UNEP) Stockholm convention (POPs Protocol)	☐ Assessment
	☐ In relevant Annex
Other processes/ EU legislation	☒ Other (provide further details below)

A risk assessment report of the substance HHCB has been prepared by the Netherlands in the context of Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances (Final version, May 2008). It has been concluded that there is no need for risk reduction measures beyond those which are being applied already (conclusion (ii)) both for the Environment and human health.

Furthermore, in the RAR published in 2008 the Netherlands concluded that HHCB does not meet the criteria for PBT substances. This point is currently under discussion based on new data available.

In addition, a compliance check has been adopted the 31st of October 2018 with the following requirements:

1. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: OECD TG 414) in a second species (rabbit), oral route with the registered substance;

2. Extended one-generation reproductive toxicity study (Annex X, Section A.7.3.i test method: OECD TG 443) in rats, oral route with the registered substance specified as follows:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation.

The requested information has to be submitted in an updated registration dossier by 7 May 2021.

Regarding other regulatory framework, HHCB has been evaluated by the SCCNFP (Scientific Committee on Cosmetic products and Non-Food Products intended for consumers) for its use as fragrance ingredient in cosmetic products (SCCNFP/0610/02, final report, 17 September 2002). SCCNFP was of the opinion that HHCB can be safely used in cosmetics without any restriction for its use. Other sources of consumer exposure from non food products (e.g. laundry products) have not been considered.

3 HAZARD INFORMATION (INCLUDING CLASSIFICATION)

3.1 Classification

3.1.1 Harmonised Classification in Annex VI of the CLP

The harmonized classification of HHCB is the following (ATP01):

- ✓ Aquatic Acute 1 (H400: Very toxic to aquatic life)
- ✓ Aquatic Chronic 1 (H410: Toxic to aquatic life with long lasting effects)
- ✓ ATP Inserted / Updated: ATP01

Classification		Labelling		
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Supplementary Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**
Aquatic Acute 1	H400			GHS09 Wng
Aquatic Chronic 1	H410	H410		

3.1.2 Self classification

- The following hazard classes are in addition notified among the aggregated self classifications in the C&L Inventory:

  Classification & Labelling notified by industry to ECHA:

  - Hazardous to the aquatic environment (acute / short-term)

  Hazard category: Aquatic Acute 1

  Hazard statement: H400: Very toxic to aquatic life.

  - Hazardous to the aquatic environment (long-term)

  Hazard category: Aquatic Chronic 1

  Hazard statement: H410: Toxic to aquatic life with long lasting effects.

3.1.3 Proposal for Harmonised Classification in Annex VI of the CLP

3.1.4 CLP Notification Status

Table: CLP Notifications

	CLP Notifications[2]
Number of aggregated notifications	15
Total number of notifiers	>1200

3.2 Human health data

Human and environmental hazard properties presented are based on available data from scientific literature and from the chemical safety report (CSR).

3.2.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

There is no data available on the toxicokinetics of HHCB after oral and inhalation exposure.

3.2.2 Acute toxicity

- Human information:

There is no data available in humans on the acute toxicity of HHCB after oral administration.

- Non-human information:

Oral route
In the 401 OECD study, an oral LD 50 value is reported to be >4.6 mg/kg/d in rats after administration of 65% HHCB in diethyl phthalate (DEP) (equivalent to >3 g/kg bw corrected dose of HHCB).

Dermal route
In the 402 OECD study, a dermal LD50 of >10.0 g/kg bw after administration of 65% HHCB in diethyl phthalate (DEP)) (equivalent to >6.5 g/kg bw corrected dose of HHCB) is reported in rabbits.

3.2.3 Repeated dose toxicity

- Human information

There is no data available in humans on the repeated dose toxicity of HHCB after oral administration.

- Non-human information:

[2] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed September 2018)

In the 90-day oral study (OCDE 408), 150 Crl:CD (SD)Br rats (5 groups of 15 males and 15 females) received HHCB (purity not reported in report) at 0, 5, 15, 50, or 150 mg/kg bw/day in the diet. Results revealed that there were no mortalities or adverse clinical signs. Body weights and food consumptions of treated groups were similar to those observed in the control group. No change in ophthalmologic evaluation was observed and no significant histopathological finding was observed at any dose.
A NOAEL of ≥150 mg/kg bw/day, the highest dose tested, for HHCB in rats is concluded.

3.2.4 Mutagenicity

HHCB has been tested in a wide array of *in vitro* tests and in an *in vivo* mouse micronucleus test. The data indicate that HHCB is a non-genotoxic substance.

3.2.5 Carcinogenicity

- Human information

There is no human data available on the carcinogenicity of HHCB.

- Non-human information

There is no experimental data available on the carcinogenicity of HHCB.

3.2.6 Toxicity for reproduction

- Human information:

There is no human data available on the carcinogenicity of HHCB.

- Non-human information:

There is no experimental data available on the carcinogenicity of HHCB.

3.2.6.1 Developmental toxicity

- Human information:

There is no information available on Human.

- Non-human information:

Only one *in vivo* study is described in the CSR. It does not meet the quality standards (large loss of body weight, one unique high dose tested,…) to be used in this report (Christian et al. 1999).

The objective of the study was to evaluate potential reprotoxic properties of HHCB in a one generation GLP study in Sprague-Dawlay rats. Pregnant rats were treated with HHCB: 50, 150, 500 mg/kg/day from GD7 –GD17 by gavage. During treatment, starting at the lowest dose, the dams suffered from body weight loss and showed multiple signs of uncomfort such as default of grooming, decreased food intake, this was accompanied by a decreased fetal bodyweight at GD20. No gross abnormalities of the different organ could be evidenced in the newborn but for, in very few cases, the apparition of a delayed ossification of the ribs and

vertebrae from the animals of the highest dose group. This study evaluated absolutely no endocrine-specific endpoints. The observations are very limited and gross. It is concluded that mothers were more sensitive than fetus but in any case it can be concluded that there was not adverse effect on the fetus with regard to the provided information.

3.3 Environment data

Not addressed in this RMOA.

3.4 Endocrine disrupting properties identification

3.4.1 Information sources and strategy for ED identification

The human and environmental studies on EDC properties analysed are based on available data from scientific literature and from the chemical safety report (CSR).

An overview of data retrieved from scientific literature search is presented in table 1. Studies were classified according to the endrocrine pathway investigated and the study type (OECD level and endpoint).

Table 1: *Overview of endpoints and studies investigating HHCB endocrine activity. Results are ordered by endocrine pathways and OECD level. The numbers represent the available results for a given endpoint. Adapted from Browne, et al. (2017).*

Endocrine Pathway	Mode of action															
	In vitro (OECD CF Level 2)											In vivo (OECD CF Level 3)				
	ER binding		ER proliferative assay	ER Transcriptional Activation		Androgen receptor activation/inhibition	Human Thyroid receptor	Steroidogenesis	TTR binding	AhR activation	Progesterone receptor activation/inhibition	Uterotrophic assay	Amphibian Metamorphosis assay	Fish test, sp. *Japanase medaka*. Aqueous exposition	Fish test, *Rainbow trout*. IP exposition	Transgenic zebrafish, aqueous exposition
	hERα	hERβ		ERα	ERβ											
E+	1	1	3	11	4							1		1	1	1
E-				7	5											1
A+						3										
A-						4										
hTR β+							1									
hTR β -							1									
Transthyretrine receptor									1							
HPT Axis													1			
Steroidogenesis (Hormonal balance)								2								

AhR activity										**1**						
PR +											**1**					
PR -											**1**					

3.4.2 Synthesis of *in vitro* information

- **Nuclear receptors**

- Estrogen receptors:

Six *in vitro* studies containing each different models as described in table 2 (Seinen et al. 1999, Schreurs et al. 2002, Schreurs et al. 2004, Gomez et al. 2005, Cavanagh et al. 2018, Schreurs et al. 2005) have conducted *in vitro* tests using various human cell lines (HEK293, HEK237, U2OS, HepG2, MCF-7, HELN and MELN), transfected or not with human ESR1 (hERα) or ESR2 (hERß), to evaluate the effect of HHCB on the activation of these receptors. All these papers agree altogether to show:
- a marginal agonist effect on ERα (LOAEC ~ 10 µM),
- no detectable agonist effect on ERß,
- an ability to antagonize the effect of estrogens on both, hERα and hERß (LOAEC = 0.1 to 1 µM).

Using HEK293 cells transiently transfected (TT) with zebrafish ER (zfER), Schreurs et al. (2004) showed that HHCB lacked agonist activity toward zfER but induced a anti-estrogenic activity on zfERß1 and zfERß2 transcriptional activity.

Using MCF7 expressing ERα, Bitsch et al. (2002) did not observed a proliferative effect of 10 µM HHCB while Evans et al. (2012) observed a weak estrogenic activity (EC10 ~ 4 µM), at a lower extent than that elicited by BPA (EC10 ~ 0.45 µM).

Table 2: *Summary of mechanistic studies related to ER activity.*

Species (receptor origin or assay)	Cell type origin	Dose range Exposure time	Observed effects	Reference
human/hERα and hERβ binding	HEK293 cells	not specified / 1hr	hERα: IC50: 21 µM hERβ: IC50: 6.1 µM	(Schreurs et al. 2002)
human / E-screen	MCF-7 cells	10µM / 120 hr	no oestrogenic activity	(Bitsch et al. 2002)
human / E-screen	MCF-7 cells	0.1 to 100 µM / 120 hr	positive activity	(Lange et al. 2014)
human / E-screen	MCF-7 cells	0.1 to 100 µM / 120 hr	positive activity	(Evans et al. 2012)
human/hERα	T47D-kbLUC	0.1 to 100 µM / 24 hr	positive activity	Evans et al. 2012)
human/ hER	MELN cells	0.1 to 10 µM / 24hr	weak positive activity	(Cavanagh et al. 2018)
human/hERα or hERβ (TT)	HepG2 cells	10 µM / 24 hr	hERα: positive for agonism (10µM), negative for antagonism hERβ: negative for agonism, positive for antagonism	(Schreurs et al. 2002)
human/hERα or hERβ	U2OS cells	0.1, 1, 10, 100 µM / 24 hr	hERα: weak agonism and antagonism hERβ: strong antagonism from 1 µM	(Schreurs et al. 2002)
human/hERalpa or hERβ	HELN cells	0.1 to 10 µM / 16 hr	hERα: weak agonism hERβ: no agonism	(Gomez et al. 2005)
human/hERα or hERβ	HEK293 cells	0.1 to 10 µM / 24 hr	hERα: no antagonism hERβ: antagonism	(Schreurs et al. 2005)
human/hERα or hERβ (TT)	HEK293 cells	0.1 , 1, 10 µM / 24 hr	hERα: no agonism, weak antagonism (at 10 µM) hERβ: no agonism, antagonism (at 0.1 µM)	(Schreurs et al. 2004)

Zebrafish/ zfERα, zfERß1, zfERß2 (TT)	HEK293 cells	0.1 , 1, 10 µM / 24 hr	zfERα: no agonism or antagonism zfERβ1: no agonism, antagonism (at 10 µM) zfERβ2: no agonism, antagonism (at 0.1 µM)	(Schreurs et al. 2004)
human/ hERα or hERβ	HEK293 cells	0.1, 1, 10, 100 µM / 24 hr	hERα: weak agonism (at 10 µM), weak antagonism hERβ: no agonism, antagonism (at 0.1 µM)	(Schreurs et al. 2002)
human/hERα or hERβ (TT)	HEK293 cells	0.1 to 50 µM / 24 hr	hERα: weak agonism hERβ: no agonism	(Seinen et al. 1999)
hamster/hERα	CHO-K1	0.0001µM to 100µM / 20 hr	weak agonistic activity antagonistic activity not detecteted	(Mori et al. 2007)
mice balb/c/ uterotrophic assay	mice balb/c	50 and 300 ppm / 2 w	No estrogenic effects on body, uterus and thymus weigths. Increase in liver weight	(Seinen et al. 1999)

- Androgen receptor:

An antagonist effect on the activation of AR was observed for high concentrations of HHCB (IC 50 = 1 µM), by using stably transfected U2OS cells (Schreurs et al. 2005). A significant antagonist effect on R1881-activated AR was also observed in PALM cells for HHCB (EC25 = 5.15 µM compared to bicalutamide), showing a greater inhibitory effect than BPA in the same conditions (EC25 = 8.1 µM) (Cavanagh et al. 2018). A positive hAR antagonist effect was also identified in Kortenkamp et al. (2014). In the same way, Mori et al. (2007) observed an AR antagonist activity by using transfected Chinese hamster ovary cells.

Table 3: *Summary of mechanistic studies related to AR activity.*

Species (receptor origin)	**Cell type origin**	**Dose range** Exposure time	**Observed effects**	**Reference**
human/hAR	PC-3 cells	0.1 to 10 µM / 24hr	Negative for agonism Positive for antagonism	(Cavanagh et al. 2018)
human/ hAR	MDA-kb2-LUC (MDA-MB-453 cell line)	0.1 to 100 µM / 24hr	Positive for antagonism	(Kortenkamp et al. 2014)
hamster/hAR	CHO-K1	0.0001µM to 100µM/ 20hr	no agonistic activity antagonistic activity	(Mori et al. 2007)
human/hAR	U2OS cells	0.1 to 10 µM / 24hr	Negative for agonism Positive for antagonism	(Schreurs et al. 2005)

- Thyroid receptor:

No TRα or TRß agonist activity was detectable neither in the stably transfected Chinese hamster ovary cells (Mori et al. 2007), nor in the T4-TTR assay (Cavanagh et al. 2018).

Table 4: *Summary of mechanistic studies related to TR activity.*

Species (receptor origin)	**Cell type origin**	**Dose range** Exposure time	Observed effects	Reference
hamster/hTRβ	CHO-K1	0.0001µM to 100µM / 20 hr	no agonistic activity antagonistic activity not	(Mori et al. 2007)

			detected EC10/EC50: not detected IC50: not detected	
transthyretrine receptor	Acellular binding	0.001 to 100 µM / 24 hr	no agonistic activity antagonistic activity.No TTR binding	(Cavanagh et al. 2018)

- Steroidogenesis:

In an adrenal human cell line, the addition of 2.5 or 25 µM HHCB in the culture medium decreases the amount of hormonal secretions and differencially modulates the mRNA expression of various steroidogenic enzymes (Li et al. 2013).

Using subcellular fractions from the gonads of Carp (*Cyprinus carpio*), it was observed that HHCB decreased the activities of various steroidogenic enzymes (IC50 equal to 68 to 1000 µM) (Schnell et al. 2009)

Table 5: *Summary of mechanistic studies related to steroidogenesis.*

Species (receptor origin)	Cell type origin	Dose range Exposure time	Observed effects	Reference
human	H295R cells	0.25, 2.5, 25 µM / 48 hr	Modulation of steroidogenesis at 25 µM - decrease in cortisol by 27% and progesterone by 39% - increase in mRNA CYP17, CYP11B1, CYP11B2 - decrease in mRNA CYP21, 3βHSD2 LOEC: 25 µM	(Li et al. 2013)
Carp *(Cyprinus carpio)*	Subcellular fraction of carp gonads	0.1 and 1 mM or 0.01, 0.1, 1, 10, and 50 µM	various steroidogenic enzymes: IC50 equal to 68 to 1000 µM	(Schnell et al. 2009)

- Other *in vitro* mechanistic pathways:

Two studies investigated other *in vitro* mechanistic pathways: one did not find AhR activity in rat hepatoma cell lines (Cavanagh et al. 2018), the other identified a hPR antagonist activity in the human osteoblastic U2OS cell line (Schreurs, Sonneveld, Jansen, et al. 2005).

Table 6*: Summary of mechanistic studies related to steroidogenesis.*

Species (receptor origin)	Cell type origin	Dose range Exposure time	Observed effects	Reference
rat/AhR	H4IIE cells	0.1 to 10 µM / 24hr	no AhR activity	(Cavanagh et al. 2018)
human/hPR	U2OS cells	0.1 to 10 µM / 24hr	- hPR antagonism activity IC50: 0.2 µM	(Schreurs et al. 2005)

Conclusion of in vitro results:

These *in vitro* studies show that HHCB has the ability to change both the activity and the expression of genes involved in steroidogenesis. However, It should be noted that many *in vitro* results were achieved at concentrations close or above the water solubility limits (6.4 µM), questioning the relevance of the observed responses.

HHCB displays an ability to activate or to antagonize steroidogenic nuclear receptors. However, these activities are generally weak and measured for high HHCB concentrations. No transcriptional activity was found for TR.

3.4.3 Synthesis of *in vivo* information

3.4.3.1 For human health

Using the uterotrophic assay, Seinen *et al.* 1999, observed an **estrogenic effect at both 0.6 and 40 mg HHCB /kg /bw**, associated to a significant and dose-dependent increase of the liver weight.

Another *in vivo* study did not meet the quality standards (large lost of body weight, one unique high dose tested…) to be used in this review (Christian et al. 1999).

Conclusions

There is only one *in vivo* test investigating this endpoint. The results do not allow to draw any firm conclusion but raise a concern.

3.4.3.2 For environmental health

- ***In vivo* mechanistic information:**

Two mechanistic studies in fish investigated the anti-estrogenic and estrogenic activities of HHCB.

Schreurs et al. (2004) reported results of an *in vivo* assay conducted with transgenic zebrafish transfected with an ERE-LUC plasmid. The 4 to 5-month old juvenile zebrafishes were exposed for 96 h to nominal concentrations of 0.01, 0.1 and 1 µM HHCB in presence of 10 nM E2. The HHCB concentrations were 15-30% lower than the expected concentration at the beginning of the experiment and, then reduced to circa 10% of the nominal concentration after 96h, either by absorption at the glass or by diffusion into the air. The purity of HHCB is not indicated. The results showed a consistent antagonistic activity of HHCB *in vivo*. Luciferase activity was reduced to 70% and 20% of the E2 positive control at 0.1 and 1 µM, respectively. The authors mentioned that HHCB had no significant estrogenic activity *in vivo*, although data were not shown. **This study indicates an ER antagonistic activity of HHCB in juvenile transgenic zebrafish and supports the anti-estrogenic activity observed *in vitro* on zebrafish ERβs transactivation in the same study.**

Yamauchi et al. (2008) assessed the estrogenic potency of HHCB in 4 month-old adult male medaka (*Oryzias latipes*) by measuring both the vitellogenin (VTG) expression by ELISA and the transcription level of selected genes by qPCR in the liver. Adults were exposed for four days to nominal doses of 5, 50 and 500 µg/L HHCB (0.02 to 2 µM, purity not mentioned) or 1 nM E2 as positive control. Mass spectrometric assays indicated effective concentrations of 4.8 ± 0.16, 49 ± 1.3, and 434 ± 18 µg/L at the start of the experiment and a 500 times reduced

concentration after 24 hours, before complete renewal of the medium. An increase in VTG protein levels was observed in the fish exposed to 500 µg/L, corresponding to 1/7 of the E2-induced level (1 nM). This induction was also observed at the transcriptional level with an increase in vgt I (at 500 µg/L) and vtg II mRNA (from 50 µg/L). Out of the genes investigated in the liver, only ERα transcription was induced at 500 µg/L. Changes in ERβ, PXR, cyp3a transcript levels were not significant. **These results convincingly indicate the existence of an estrogenic activity of HHCB in male medaka.**

Conclusion:

Two mechanistic studies in fish investigated the effect of HHCB on ER signaling pathway (Schreurs et al. 2004, Yamauchi et al. 2008). The study by Schreurs *et al*. (2004) showed an *in vivo* anti-estrogenic activity of HHCB using a juvenile transgenic zebrafish model, in agreement with the antagonist activity observed on zebrafish ERß transactivation *in vitro*. Yamauchi *et al*. (2008) observed an induction of vtg genes expression and VTG protein in adult male medaka, indicative of an agonist estrogenic activity. These results indicate a potential interference of HHCB on ER signaling in fish with both, agonist and antagonist activities that may vary between species or development stages.

- ***In vivo* growth, reproductive and developmental toxicities:**

- <u>Vertebrates:</u>

Pablos et al. (2016) examined the effects of the musk HHCB on *Xenopus laevis* amphibian model using a protocol adapted from the OECD TG 231. The authors exposed premetamorphic tadpoles *via* food to four various concentrations of HHCB: 0.05mg/kg; 0.5mg/kg; 5mg/kg and 50mg/kg. The authors examined growth parameters at day 14 and day 23 after exposure. They reported a transient developmental acceleration for the group exposed at 50 mg/kg at day14, which was not observed at day 23. Histological parameters of the thyroid gland were investigated at day 23 (tadpoles) and at the end of metamorphosis. At both stages, thinner follicle cell epithelia were seen for the 5 and 50 mg/kg exposed groups. Papillary projections have been reported at the day 23 for the two highest doses. Some limitations were noted, notably the lack of a statistic analysis of histopathologic data as well as the lack of thyroid hormone measurements to support the hypothesis of a thyroid-related effect. **The major concern came from internal HHCB measurements done in total froglets. Information was missing in the manuscript and questioned the validity of the entire work. The corresponding author did not provide clarification; therefore, we dismissed this publication for the evaluation of HHCB EDC effect.**

- <u>Invertebrates :</u>

Ramskov et al. (2009) report results from an experiment in which worms (*Capitella sp*.) were exposed to sediment contaminated with HHCB (1.5, 26, 123 and 168 mg/kg dry weight (dw) –controlled by analytical procedure). Experimental conditions were in agreements with previous data indicating favourable conditions for the culture of the worms. HHCB was of good quality with 98% purity. Experimental design was appropriate.

Juvenile survival was significantly reduced at 123 and 168 mg/kg HHCB (declined by 26 and 29% respectively). Maturation time was impaired at 168 mg/kg. In contrast, HHCB had no significant effect on juvenile growth. A dose-dependent decrease in the percentage of male worms was observed from 26 mg/kg in treated groups (50% in control vs 39% in the highest concentration tested), which lead to an increase in number of hermaphrodites. A dose-dependent effect was observed on egg production (significant at 26 mg/kg), the number of brood (non-significant) and brood size (significant at 123 mg/kg) from the lowest dose tested. Population analysis lead to observe a declining trend for population growth rate with increasing HHCB concentrations. **This study shows that HHCB can impair reproductive and developmental parameters in *Capitella sp.* and raise the question on any endocrine action leading to change in sex-ratio.**

Pedersen et al. (2009) report results from an experiment in which gastropods (*Potamopyrgus antipodarum*) were exposed to sediment contaminated with HHCB (0.1, 1, 10, 30 and 100 mg/kg dw –controlled by analytical procedure). The purity of HHCB was not reported, but the experimental setup is the same as that described by Ramskov, 2009. The experimental conditions were in agreements with the previous data indicating favourable conditions for the culture of the molluscs. This experimental design was appropriate. The juveniles were exposed from birth to first reproduction to measure juvenile survival, growth, time to first reproduction and size at first reproduction. The adults were exposed for 12 weeks to measure growth rate and reproduction.

Adult survival was not affected by HHCB, but effects on juveniles were observed (90% survival at 30 mg/kg and 80% at 100 mg/kg). Similarly, growth rate was affected in juveniles (from 30 mg/kg) and the time to first reproduction was significantly elevated at 100 mg/kg. Total number of offspring was reduced in a dose-dependent and significant manner from 10 mg/kg. A population model indicated a dose-dependent, but not significant, decrease (by ca. 2%), on population growth rate under otherwise favourable laboratory conditions. **This study shows that HHCB can impair reproductive and developmental parameters in *P. antipodarum***.

Conclusion on invertebrates

Two articles from the invertebrate literature indicate that HHCB has had significant reproductive and developmental effects on tested molluscs and worms by decreasing egg and offspring production and time to reproduction of the offsprings (Ramskov et al. 2009, Pedersen et al., 2009). These results are corroborated by the reproductive and developmental toxicity data provided in the CSR. **Together these results indicated consistant effects on representatives of several invertebrate groups including arthropods, molluscs and worms.** These effects may be environmentally relevant, especially because the laboratory conditions gave suboptimal growth conditions.

However, it should be noted that there is so far no guidance on how to use/ interpret invertebrates data in the ED identification.

3.4.4 Analysis of the evidence and conclusion on ED properties

Data on endocrine properties of HHCB in vertebrates is limited to some *in vitro* and *in vivo* mechanistic studies (OECD level 2 and 3).

An endocrine activity has been observed on ER pathway. Several *in vitro* studies report a weak agonist activity on ERα and an antagonist activity on ERβ. In short-term exposure tests performed on fish, HHCB induced the VTG mRNA expression in adult male medaka, indicating an estrogenic effect. In contrast, only anti-estrogenic activity of HHCB was observed in juvenile ERE-luciferase transgenic zebrafish exposed for 96 h. The *in vivo* results support these *in vitro* findings, and highlight the differential capacity of HHCB to interfere with ER signalling. Albeit an alert on ER signalling could be identified, information on HHCB effects on reproduction and development of the fish would be needed to draw a firm conclusion about the HHCB (anti)estrogenic effects.

Regarding other signaling pathways, the available data are not sufficient to conclude. There is no sufficient information on AR signaling pathway. There is no alert on TR signalling pathway, but additional studies would be required to conclude. In addition, an alert on steroidogenic activity has been identified, but information are requested to confirm the observed effect.

Regarding the vertebrate toxicity related to human and environmental health, there is a lack of information on reproductive and developmental toxicity. Based on the one *in vivo* test measuring only one endpoint (Seinen *et al*., 1999), it is not possible to conclude whether or not HHCB displays endocrine adverse effects.

Contrasting with vertebrates, the reproductive toxicity of HHCB on invertebrates has been evidenced in several studies. These studies highlight the possible ED properties of HHCB identified in arthropods, worms and molluscs. However, based on current knowledge, no biological plausible link can be established between a reproductive adverse effect in invertebrates and an endocrine mode of action, as this is required for the identification of an ED based on the EU definition and criteria (Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009).

In this context, further investigations are needed, particularly on long-term reproductive and developmental toxicity in vertebrates (rodent and/or fish) and on endocrine mechanisms in invertebrate species, to assess the ED properties of HHCB. A compliance check (CCH) is currenlty proposed by ECHA, which requires a pre-natal developmental toxicity study (OECD TG 414) and an extended one-generation reproductive toxicity study (OECD TG 443), with cohorts 1A and 1B (with extension to mate the Cohort 1B animals to produce the F2 generation). These studies may provide useful information to state on the ED long-term effects for human health. Depending on the outcomes of the CCH, and after evaluating the new dataset, other studies could be considered. Further work on environmental health within the Corap would be necessary to clarify the concern on endocrine effects in fish.

4 INFORMATION ON (AGGREGATED) TONNAGE AND USES[3]

4.1 Tonnage and registration status

Table: Tonnage and registration status

From ECHA dissemination site	
Registrations	☒ Full registration(s) (Art. 10) ☐ Intermediate registration(s) (Art. 17 and/or 18)
Total tonnage band for substance (excluding volume registered under Art 17 or Art 18, or directly exported)	1,000-10,000 tpa

4.2 Overview of uses

This substance is used in the following products: biocides (e.g. disinfectants, pest control products), washing & cleaning products, air care products, polishes and waxes, perfumes and fragrances and cosmetics and personal care products. *Information available from other open sources relevant for this case can also be included.*

Table: Uses

	Use(s)
Uses as intermediate	
Formulation	Formulation of dertergents and maintenance products Formulation of fragrance products (cosmetics)
Uses at industrial sites	Industrial use of washing and cleaning products Industrial use of dertergents and maintenance products

[3] *Please provide here the date when the dissemination site was accessed.*

Uses by professional workers	Polishes and wax blends Detergents and maintenance products
Consumer Uses	Cosmetics, air care products, washing and cleaning products, biocides
Article service life	

4.3 Additional information

HHCB is the largest volume product of the fragrance materials known collectively as polycyclic musks. Fragrance oils are complex mixtures, prepared by blending many fragrance ingredients in varying concentrations. Most of these ingredients are liquids, in which HHCB is mixed. Applications of the fragrance oils are in consumer products such as perfumes, cosmetics, soaps, shampoos, detergents, fabric conditioners, household cleaning products, air fresheners etc. (EU RAR, 2008).

5 JUSTIFICATION FOR THE RISK MANAGEMENT OPTION

5.1 Need for (further) risk management

Table: SVHC Roadmap 2020 criteria

	Yes	No
a) Art 57 criteria fulfilled?		X?
b) Registrations in accordance with Article 10?	x	
c) Registrations include uses within scope of authorisation?	x	
d) Known uses not already regulated by specific EU legislation that provides a pressure for substitution?	x	

Previous assessments of the substance HHCB have not identify the need to implement risk reduction measures beyond those which are being applied already (RAR Final version, the Netherlands, May 2008) and the substance can be safely used in cosmetics without any restriction for its use (SCCNFP/0610/02, final report, 17 September 2002).

However, in the framework of the French National Strategy on Endocrine Disruptors in 2018 and considering REACH registration data, the French Competent Authority requested Anses to evaluate the ED properties of HHCB.

Anses considers that further investigations are needed, particularly on long-term reproductive and developmental toxicity in vertebrates (rodent and/or fish) and on endocrine mechanisms in invertebrate species, to assess the ED properties of HHCB.

Furthermore, in the RAR written in 2009 the Netherlands concluded that HHCB does not meet the criteria for PBT substances. This point is currently under discussion based on new methods and data available. No conclusion is made at this stage.

5.2 Conclusions on the most appropriate (combination of) risk management options

The substance case has been discussed at EDEG-13 meeting.

A compliance check (CCH) has been issed by ECHA the 31st of October 2018, which requires a pre-natal developmental toxicity study (OECD TG 414) and an extended one-generation reproductive toxicity study (OECD TG 443), with cohorts 1A and 1B (with extension to mate the Cohort 1B animals to produce the F2 generation). These studies may provide useful information to state on the ED long-term effects for human health. Depending on the outcomes of the CCH, and after evaluating the new dataset, other studies could be required. Further work on environmental health within the Corap would be necessary to clarify the concern on endocrine effects in fish.

Regarding PBT properties, even if the Netherlands concluded that HHCB does not meet the criteria for PBT substances (RAR 2008), there is a need to reassess based on new methods and data available. No conclusions is made a this stage by FR-MSCA.

5.3 References

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