Centre on Endocrine Disrupters

Assessment of the endocrine disrupting potential of 23 UV-filters (j.no. MST-656-00150)

DANISH CENTRE FOR ENDOCRINE DISRUPTERS

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1. Terms of reference and scope

This report has been prepared by the Danish Centre on Endocrine Disrupters (CEHOS) as a project contracted by the Danish Environmental Protection Agency. The Danish Centre on Endocrine Disrupters is an interdisciplinary scientific network without walls. The main purpose of the Centre is to build and gather new knowledge on endocrine disrupting chemicals (EDCs) with the focus on providing information requested for the preventive work of the regulatory authorities. The Centre is financed by the Ministry of the Environment and the scientific work programme is followed by an international scientific advisory board.
The overall scope of this project is to provide a science based evaluation of 23 UV-filters with regard to their endocrine disrupting potential.

2. Background and aim

The Danish EPA has asked the Danish Centre on Endocrine Disrupters to evaluate all UV-filters allowed for use in Europe for their endocrine disrupting potential. Since 7 of these UV-filters have previously been assessed for their endocrine disrupting potential, according to the Danish criteria for EDCs, and these assessments have been published in a report from the Danish Centre on Endocrine Disrupters (Hass *et al.*, 2012) the following seven UV-filters were not assessed in the present report: Benzophenone 1, 2 and 3 (BP1, 2 and 3); dihydroxybenzophenone, 4-metylbenzylidene camphor (4MBC), 3-benzylidene camphor (3-BC); ethylhexyl methoxycinnamate (OMC). The overall aim of the present project was to gather all accessible knowledge on the potential endocrine disrupting properties of the remaining 23 UV-filters, shown in table 1.

Table 1. The evaluated UV-filters (INCI names)

BENZOPHENONE-4	DIETHYLAMINO HYDROXYBENZOYL HEXYL BENZOATE	ETHYLHEXYL TRIAZONE	PHENYLBENZIMIDAZOLE SULFONIC ACID
BENZOPHENONE-5	DIETHYLHEXYL BUTAMIDO TRIAZONE	HOMOSALATE	POLYACRYLAMIDOMETHYL BENZYLIDENE CAMPHOR
BENZYLIDENE CAMPHOR SULFONIC ACID	DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE	ISOAMYL P-METHOXYCINNAMATE	POLYSILICONE-15
BIS-ETHYLHEXYLOXYPHENOL METHOXYPHENYL TRIAZINE	DROMETRIZOLE TRISILOXANE	METHYLENE BIS-BENZOTRIAZOLYL TETRAMETHYLBUTYLPHENOL	TEREPHTHALYLIDENE DICAMPHOR SULFONIC ACID
BUTYL METHOXYDIBENZOYL METHANE	ETHYLHEXYL DIMETHYL PABA	OCTOCRYLENE	TITANIUM DIOXIDE
CAMPHOR BENZALKONIUM METHOSULFATE	ETHYLHEXYL SALICYLATE	PEG-25 PABA	

3. Methods

3.1 Literature

Generally, the literature used for evaluation of the substances aimed to comprise all relevant publicly available knowledge on reproductive and endocrine effects of the investigated UV-filters. Therefore results from scientific papers including both *in vitro* and *in vivo* studies, studies in the environment, and all relevant information published in the European SCCS (Scientific Committee on Consumer Safety) opinions was included in the evaluation.

A list of UV-filters allowed in cosmetic products and the SCCS opinions were found on the following web-address:

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.results&annex_v2=VI&search

For the *in vitro* and *in vivo* toxicity studies related to human health, the open literature search was done in MEDLINE using relevant and similar search criteria for each substance. The search was primarily done using the INCI name of the substance, but also included searches on the chemical name, the CAS number and other commonly used substance names. For example, for ethylhexyl dimethyl paba, the MEDLINE search also included the following synonyms: 2-ethylhexyl 4-(dimethylamino)benzoate; Padimate O; octyl dimethyl paba; and OD-PABA. If many hits were present in the MEDLINE search for the substance name alone, the following searches phrases were included: endocrine*, toxicity*, estrogen*, androgen*, thyroid*, for each synonym.
For the ecotoxicity studies the open literature search was done in Web of Knowledge searching in all databases: Web of Science, MEDLINE and Journal Citation Reports. The search included the INCI name of the substance, the chemical name and other commonly used substance names. The search on titanium dioxide alone resulted in a vast amount of hits and therefore included the search terms: endocrine, ecotoxicity, oestrogen*, estrogen*, androgen*, aromatase or thyroid*.

In a few cases, additional literature search based on references in the retrieved papers was performed.

3.2 Evaluation

Evaluations of SCCS opinions and of *in vitro* and *in vivo* toxicity studies related to human health from the open literature, were done at DTU (National Food Institute, Technical University of Denmark), whereas evaluations of ecotoxicity data were done at SDU (Institute of Biology, University of Southern Denmark). This was followed by an overall evaluation in the project group.

4. Results and discussion

4.1 Results from assessment of human health

For the majority of the investigated UV-filters, very little information on the endocrine disrupting potential of the compounds could be found. For 5 of the compounds no SCCS opinions were available, and for 6 others no information on reproductive toxicity was present in the opinions. Short opinions, where reproductive toxicity results were summarized in one line were present for 8 of the compounds, whereas somewhat more detailed study summaries dealing with reproductive toxicity or potential endocrine mode of action were present for the remaining 4 compounds. In the open literature search, no relevant literature dealing with reproductive toxicity or endocrine disruption was found for 13 of the compounds, whereas relevant literature was present for 10 of the compounds.
Based on both SCCS opinions and the open literature, a division of the 23 UV-filters with regards to the amount of available information and severity of reproductive and endocrine effects, was performed.

For 6 of the 23 compounds [benzophenone-5; benzylidene camphor sulfonic acid, diethyhexyl butamido triazone, polyacrylamidomethyl benzylidene camphor, polysilicone-15 and terephtalylidene dicamphorsulfonic acid] (seen in table 2), no information on reproductive toxicity or endocrine disrupting potential was available from opinions, either because no opinions were available or because reproductive toxicity results or conclusions were not included in the opinions. Furthermore no relevant studies on these compounds were present in the open literature.

Table 2. Compounds with no information on endocrine properties or reproductive toxicity

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products*	Open litera-ture	SCCS opinion	Assessment of reproductive toxicity in opinion
BENZOPHENONE-5	6628-37-1	Benzenesulfonic acid, 5-benzoyl-4-hydroxy-2-methoxy-, monosodium salt	5% (as acid)	-	Short opinion from 1999	not included in opinion
BENZYLIDENE CAMPHOR SULFONIC ACID	56039-58-8	alpha-(2-Oxoborn-3-ylidene)toluene-4-sulphonic acid and its salts	6% (as acid)	-	no available opinion, substance approved in 1994	
DIETHYLHEXYL BUTAMIDO TRIAZONE	154702-15-5	Benzoic acid, 4,4-{[6-[[[(1,1-dimethylethyl)amino]carbonyl]phenyl]amino]-1,3-5-triazine-2,4-diyl]diimino}bis-, bis(2-ethylhexyl)ester / Iscotrizinol	10%	-	Short opinion from 1997	not included in opinion
POLYACRYLAMIDOMETHYL BENZYLIDENE CAMPHOR	113783-61-2	Polymer of N-{(2 and 4)-[(2-oxoborn-3-ylidene)methyl]benzyl}acrylamide	6%	-	no available opinion, substance approved in 1996	
POLYSILICONE-15	207574-74-1	Dimethicodiethylbenzalmalonate	10%	-	Comprehensive opinion from 2010	Two generation: no data Teratogenicity: no data
TEREPHTHALYLIDENE DICAMPHOR SULFONIC ACID	92761-26-7 / 90457-82-2	3,3'-(1,4-Phenylenedimethylene) bis (7,7-dimethyl-2-oxobicyclo-[2.2.1] hept-1-ylmethanesulfonic acid) and its salts / Ecamsule	10% (as acid)	-	no available opinion, substance approved in 1994	

***According to Cosmetics directive 76/768/EEC, annex VII**

For the UV-filters shown in table 3 [Benzophenone-4 (BP4) & Octisalate], short opinions were available, but in these no information on reproductive toxicity was presented. BP-4 had been tested for binding to the androgen receptor by Ma *et al.* (2003), but they found no affinity to this receptor. The toxicity of octisalate was reviewed by both the Cosmetic Ingredient Expert Panel (2003) and by Lapczynski *et al.* (2007) but like in the SCCS opinion, no data regarding reproductive and developmental toxicity were presented in any of these publications. Furthermore, octisalate was investigated for estrogenic activity *in vitro* by Morohoshi *et al.* (2005) and here the UV-filter showed weak estrogenic activity. However, without any *in vivo* data on reproductive development or function, it is difficult to conclude anything on the endocrine disrupting potential of octisalate, based on this one study.

Table 3. UV-filters with no information on endocrine properties or reproductive toxicity from opinions and with only *in vitro* data from the open literature.

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products	Open literature	SCCS opinion	Assessment of reproductive toxicity in opinion
BENZOPHENONE-4;	4065-45-6	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid (Benzophenone-5) and its sodium salt / Sulisobenzone	5% (as acid)	BP-4 was tested for binding to the androgen receptor *in vitro*, but showed no agonism or antagonism (Ma *et al.* 2003).	Short opinion from 1999	not included in opinion
ETHYLHEXYL SALICYLATE	118-60-5	2-Ethylhexyl salicylate / Octisalate	5%	Ethylhexyl salicylate was shown to have weak estrogenic activity in vitro (Morohoshi *et al.* 2005). Lapczynski *et al.* (2007) and the Cosmetic Ingredient Expert Panel (2003) reviewed toxicological data on the substance but no data regarding reproductive and developmental toxicity were presented in any of these publications.	Short opinion from 1995	not included in opinion

For 4 of the compounds (seen in the top part of table 4) [ethylhexyl triazone; disodium phenyl dibenzimidazole tetrasulfonate; drometrizole trisiloxane; peg-25 paba], the only information on reproductive toxicity or endocrine disrupting potential was from short SCCS opinions, where only conclusions regarding embryotoxicity e.g. "no evidence of teratogenicity" were available. Since these conclusions were present in the opinions, at least some sort of reproductive *in vivo* testing had been performed on these 4 compounds, but for most of the compounds this probably constituted a prenatal developmental toxicity study (earlier known as teratology study), in which pregnant dams were dosed with the compounds during gestation, and the offspring were assessed for organ and skeletal malformations on the day before birth, yielding very little information on the endocrine disrupting potential of the chemicals. For the fifth compound in table 4 [diethylamino hydroxybenzoyl hexyl benzoate], a more comprehensive opinion was present, but the conclusion was rather similar to those of the other compounds in the table, i.e. that no signs of teratogenicity were seen. In the open literature no relevant information for these five UV filters was found.

Isoamyl p-methoxycinnamate (table 5) only had a short SCCS opinion, and its conclusion was that there were no signs of teratogenicity within reasonable dose ranges. In the open literature a

teratogenicity study was found. Here pregnant rat dams (n=20) were dosed with 250, 750 and 2000 mg/kg/day during gestation. The high dose caused marked maternal toxicity and increased embryonic deaths whereas some degree of maternal toxicity was seen in the group dosed with 750 mg/kg/day, but no signs of teratogenic anomalities were seen in the offspring in the two low doses. An oral dose of 250 mg/kg/day was regarded as a safe NOAEL (Jekat *et al.* 1992).

Table 4. UV-filters with limited information on reproductive toxicity from opinions, and no data from the open literature

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products	Open litera-ture	SCCS opinion	Assessment of reproductive toxicity in opinion
ETHYLHEXYL TRIAZONE	88122-99-0	2,4,6-Trianilino-(p-carbo-2'-ethylhexyl-1'-oxy)-1,3,5-triazine / octyl triazone	5%	-	Short opinion from 1996	No signs of embryotoxicity or teratogenicity within reasonable dose ranges
DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE	180898-37-7	Sodium salt of 2,2'-bis(1,4-phenylene)-1H-benzimidazole-4,6-disulfonic acid / Bisdisulizole disodium (USAN)	10% (as acid)	-	Short opinion from 1999	No evidence of teratogenicity
DROMETRIZOLE TRISILOXANE	155633-54-8	Phenol, 2-(2H-Benzotriazol-2-yl)-4-Methyl-6-(2-Methyl-3-(1,3,3,3-Tetramethyl-1-(Trimethylsilyl)Oxy)-Disiloxanyl)Propyl	15%	-	Short opinion from 1997	No signs of embryotoxicity or teratogenicity within reasonable dose ranges
PEG-25 PABA	116242-27-4	Ethoxylated Ethyl-4-Aminobenzoate	10%	-	Short opinion from 1997	No teratogenicity in a not validated alternative test
DIETHYLAMINO HYDROXYBENZOYL HEXYL BENZOATE	302776-68-7	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-,hexylester	10%	-	Comprehensive opinion from 2008	No two generation study was performed. In the teratogenicity study there were no substance-induced, dose related influences on the gestational parameters and no signs of prenatal developmental toxicity, especially no substance induced indications of teratogenicity, up to and including the highest dose level (1000 mg/kg bw/d).

Table 5. UV-filter with very limited information on reproductive toxicity from opinions, but some data from the open literature

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products	Open literature	SCCS opinion	Assessment of reproductive toxicity in opinion
ISOAMYL P-METHOXYCINNAMATE	71617-10-2	Isopentyl-4-methoxycinnamate / Amiloxate	10%	Isoamyl p-methoxycinnamate did not cause any teratogenic anomalities in the offspring up to doses of 750 mg/kg/day (Jekat *et al.* 1992).	Short opinion from 1997	No signs of embryotoxicity or teratogenicity within reasonable dose ranges

For all six UV-filters in tables 4 & 5, the available information was however not very informative in relation to the endocrine disrupting potential of the chemicals, as no endpoints sensitive to

endocrine disruption were investigated in these studies, and if they were, they are not reported in the opinion summaries or in the publication. So for 14 of the 23 compounds, it is impossible to say anything conclusive about their endocrine disrupting potential, because data is lacking.

For a large part of the remaining UV-filters there are *in vitro* studies, testing the compounds' binding affinity to different steroid hormone receptors (i.e. estrogen-, androgen- and progesterone receptors), as well as *in vivo* screening tests for estrogenic activity i.e. uterotrophic assays.

Table 6. UV-filters showing no binding to steroid hormone receptors in *vitro* and no uterotrophic effect *in vivo*

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products	Open literature	SCCS opinion	Assessment of reproductive toxicity in opinion
CAMPHOR BENZALKONIUM METHOSULFATE	52793-97-2	N,N,N-Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulphate	6%	-	Comprehensive opinion from 2006	No *in vitro* assays. Uterotrophic assay showed a slight decrease in the uterus weight. In the group receiving EE, the uterus weights were significantly higher than the control group values. The changes were considered to bear no toxicological significance. Teratogenicity: Caesarean data showed no teratogenic effects.
BIS-ETHYL HEXYL OXY PHENOL METHOXY PHENYL TRIAZINE	187393-00-6	2,2'-(6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-diyl)bis(5-((2-ethylhexyl)oxy)phenol) / Bemotrizinol / Tinsorb S	10%	No competition with E2 in the ER competitive binding assay or with R1881 in the AR competitive binding assay, thus indicating absence of estrogenic and androgenic activity. Tinosorb S was inactive in the immature rat uterotrophic assay (Ashby *et al.* 2001).	Short opinion from 1998	No evidence of teratogenicity
METHYLENE BIS BENZO TRIAZOLYL TETRA METHYL BUTYL PHENOL	103597-45-1	2,2'-Methylenebis(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol) / Bisoctrizole /Tinsorb M	10%	No competition with E2 in the ER competitive binding assay or with R1881 in the AR competitive binding assay, thus indicating absence of estrogenic and androgenic activity. Tinosorb M was inactive in the immature rat uterotrophic assay (Ashby *et al.* 2001).	Short opinion from 1999	No teratogenic potential observed
PHENYL BENZIMIDAZOLE SULFONIC ACID	27503-81-7	2-Phenylbenzimidazole-5-sulphonic acid and its potassium, sodium and triethanol amine salts / Ensulizole	8% (as acid)	-	Comprehensive opinion from 2006	In vitro: no affinity for the ER or the AR-receptors. In vivo: No effect on uterus size in uterotrophic assay in immature rats. Prenatal developmental toxicity: no effects seen on implantations, resorptions, foetus weights or malformations.

For the first compound in table 6, camphorbenzalkonium methosulfate, no studies were present in the open literature, but the SCCS opinion included somewhat detailed results from the industry studies. No *in vitro* studies were performed, and an uterotrophic study showed a slight decrease in the uterus weight, reaching a statistical significance level at 100 mg/kg/day. In the group receiving EE, the uterus weights were significantly higher than the control group values and the minimal and non-dose related changes in uterus weight were considered to bear no toxicological significance. In

the performed teratology studies doses of 100, 300 and 1000 mg/kg/day were used and Caesarean data, including pre- and post- implantation loss, foetal weight, uterus weight and foetal sex ratio, did not show any treatment-related changes. Examination of the foetuses did not reveal any treatment-related abnormalities, including teratogenic effects under the conditions of the study. This indicates that the lowered uterine weight seen in the uterotrophic study probably was a chance finding rather that e.g. an anti-estrogenic effect.

For the tree compounds shown in the bottom part of table 6 [bisethylhexyloxyphenol methoxyphenyltriazine; methylene bisbenzotriazolyltetramethylbutylphenol; phenylbenzimidazole sulfonic acid] data from either the open literature studies or SCCS opinions showed that *in vitro* these UV-filter showed no binding to the estrogen- or androgen receptors, and uterotrophic assays showed no estrogenic effects *in vivo*. For the first two UV-filters, also called Tinsorb S and Tinsorb M respectively, only short opinions existed, in which reproductive study results were summarized in a few words, however a study by Ashby *et al.* (2001) tested the two compounds for their endocrine disrupting potential. Both compounds failed to compete with either E2 in the ER competitive binding assay or with R1881 in the corresponding AR competitive binding assay, thus indicating the absence of intrinsic (agonist or antagonist) estrogenic and androgenic activity. Both compounds were also inactive in the immature rat uterotrophic assay at the tested dose levels (250, 500 and 1000 mg/kg/day).
No information on phenylbenzimidazole sulfonic acid was found in the open literature, but the SCCS opinion included data on reproductive toxicity and endocrine disrupting potential. The *in vitro* studies included the estrogen (ER)-binding screen, using human recombinant ER α-subtype as receptor and radiolabelled estradiol as ligand. The results showed that the test article did not replace labeled ligand from the ER. In the androgen receptor (AR)-binding screen the test compound showed no affinity for the AR-receptor. *In vivo* estrogenic effects were tested in an uterotrophic assay in immature rats. No effect on uterus size was seen at 50 or 200 mg/kg bw/day after 3 days of dosing. A prenatal developmental toxicity was also performed showing no maternal toxicity at 1000 mg/kg/day and no effects on implantations, resorptions, foetus weights or malformations (SCCS opinion on phenylbenzimidazole sulfonic acid, 2006).

For the 3 UV-filters shown in table 7 [butyl methoxydibenzoylmethane; ethylhexyl dimethyl PABA; homosalate] *in vitro* data indicating possible endocrine disrupting potential are present, however no adverse effects have been seen *in vivo* in uterotrophic assays. For all three UV-filters, androgenic/antiandrogenic activity has not been investigated *in vivo.*
Butyl methoxydibenzoylmethane, had no available SCCS opinion, however some data on the endocrine disrupting potential of this compound were found in the open literature. No activation of MCF7 cells was seen by Schlumpf *et al.* (2001), but weak ERα agonism was seen in another study (Schreurs *et al.* 2005). AR antagonism was shown by Schreurs *et al.* (2005), whereas Ma *et al.* (2003) did not find androgen receptor binding, and no progesterone receptor antagonism was seen (Schreurs *et al.* 2005). Furthermore, no adverse effects were found in the uterotrophic assay performed by Schlumpf *et al.* (2001).

Table 7. UV-filters showing receptor binding in some *in vitro* assays but no uterotrophic effect *in vivo*

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products	Open literature	SCCS opinion	Assessment of reproductive toxicity in opinion:
BUTYL METHOXY DIBENZOYL METHANE	70356-09-1	1-(4-tert-Butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione / Avobenzone / B-MDM	5%	No progesterone receptor antagonism, but weak ERα agonism and AR antagonism (Schreurs *et al.* 2005). Ma *et al.* 2003 did not find androgen receptor binding, and the compound showed no activation of MCF7 cells and no effect in uterotrophic assay (Schlumpf *et al.* 2001).	no available opinion, substance approved in 1993	-
ETHYL HEXYL DIMETHYL PABA	21245-02-3	2-Ethylhexyl 4-(dimethylamino)benzoate / Padimate O (USAN:BAN) /OCTYL DIMETHYL PABA / OD-PABA / Et-PABA	8%	OD-PABA has been shown to have estrogenic effects *in vitro* (Schlumpf *et al.*2001; Gomez *et al.* 2005; Schreurs *et al.* 2002; Kunz & Fent 2006; Morohoshi *et al.* 2005). Androgenic activity *in vitro* has been reported in some studies (Kunz & Fent 2006) but not in others (Ma *et al.* 2003). *In vivo* OD-PABA showed no uterotrophic effect in immature rats (Schlumpf *et al.* 2001).	Short opinion from 1999	No evidence of teratogenic activity, no embryo-toxicity.
HOMOSALATE	118-56-9	Benzoic acid, 2-hydroxy-, 3,3,5-trimethylcyclohexyl ester / Homosalate	10%	Homosalate has been shown to have estrogenic effects *in vitro* (Schlumpf *et al.* 2001; Gomez *et al.* 2005; Schreurs *et al.* 2002, 2005; Kunz & Fent 2006), and bind to the progesterone receptor (Schreurs *et al.* 2005). Androgen receptor antagonism *in vitro* has also been reported (Ma *et al.* 2003; Kunz & Fent 2006). *In vivo* homosalate showed no uterotrophic effect in immature rats (Schlumpf *et al.* 2001).	Comprehensive opinion from 2007	There are no studies available with homosalate in respect to reproductive performance or developmental toxicity including teratogenicity. However, its metabolites are comprehensively investigated in respect to teratogenicity and no adverse effects have been revealed. Industry studies investigating estrogenic and androgenic effects of homosalate showed no binging to the ER *in vitro*, weak affinity to the AR (which was interpreted as not being a specific interaction with the AR) and *in vivo* no uterotrophic effect in doses up to 1000 mg/kg/day.

In the short SCCS opinion, which was available on ethylhexyl dimethyl PABA, no evidence of embryotoxicity was concluded. In the open literature, OD-PABA has been shown to cause increased MCF7 cell proliferation (Schlumpf *et al.* 2001), increased transactivation of hERα (Gomez *et al.* 2005; Schreurs *et al.* 2002) and antagonism of ERα (Kunz & Fent 2006; Morohoshi *et al.* 2005). On the other hand, no antagonism on ERα or β was reported by Schreurs *et al.* (2002). Furthermore, no agonistic action on hERα or β has been reported (Morohoshi *et al.* 2005, Schreurs *et al.* 2002, Kunz *et al.* 2006, Kunz & Fent 2006). Androgenic activity *in vitro* has been seen as antagonism of hAR transactivation by Kunz & Fent (2006), however not by others (Ma *et al.* 2003) and no agonistic action on hAR tranactivation has been reported (Kunz & Fent 2006, Ma *et al.* 2003, Schreurs *et al.* 2005). *In vivo* OD-PABA showed no uterotrophic effect in immature rats (Schlumpf *et al.* 2001).

For homosalate, the comprehensive SCCS opinion included no studies dealing with reproductive performance or developmental toxicity of the compound, including no teratogenicity data. However, it was concluded that since the metabolites of homosalate are comprehensively investigated with respect to teratogenicity, and no adverse effects have been revealed, there was no need for further studies. The opinion also included data from industry studies investigating estrogenic and androgenic effects of homosalate. Here no binding to the ER *in vitro*, weak affinity to the AR (which was interpreted as not being a specific interaction with the AR) and no uterotrophic effect in doses up to 1000 mg/kg/day were seen. In the open literature, homosalate has also been investigated for endocrine disrupting properties. Here, homosalate was shown to have estrogenic effects *in vitro*, as increased MCF7 cell proliferation (Schlumpf *et al.* 2001), increased transactivation of hERα (Gomez *et al.* 2005, Schreurs *et al.* 2002, 2005) and antagonism of hERα (Kunz& Fent 2006) was reported. On the other hand no antagonism on ERα or β was seen by Schreurs *et al.* (2002). No agonistic action on hERα has been reported (Kunz *et al.* 2006, Kunz & Fent 2006). Androgenic activity *in vitro* in the form of antagonism of hAR transactivation has been seen by Ma *et al.* (2003) and Kunz & Fent (2006), who also found agonistic action (Kunz & Fent 2006), whereas no agonistic action on hAR transactivation was reported by Schreurs *et al.* (2005). Schreurs *et al.* (2005) also found antagonism of progesterone receptor transactivation.
In vivo homosalate showed no uterotrophic effect in immature rats (Schlumpf *et al.* 2001).

The conflicting results regarding binding to the estrogen- and androgen receptors activity indicate that the agonism/antagonism may be partial. Furthermore the studies differ in their type of assay and in some cases the tested concentrations, which may explain why some studies have found certain effects which have not been repeated in others.

All in all, for the 7 compounds found in tables 6 and 7, uterotrophic tests have been performed, and regardless of the UV-filters' abilities to bind to the estrogen receptor, no adverse uterotrophic effects have been seen, indicating lack of *in vivo* estrogenic potential for these compounds. Only some of the compounds have been investigated for AR agonism/antagonism *in vitro*, and as mentioned above, the results differ somewhat depending on which type of study has been performed. Since no *in vivo* studies investigating the antiandrogenic effects of the compounds have been performed, it is difficult to conclude anything on their endocrine disrupting potential with regard to androgenic/antiandrogenic mode of action.

The last two compounds included in this evaluation [octocrylene; titanium dioxide] are shown in table 8. For both compounds no data on endocrine disrupting mode of action *in vitro* or *in vivo* exist, but the compounds are placed together in this table because some developmental studies regarding their reproductive and developmental toxicity exist, and some data indicate that one of the compounds could be a reproductive toxicant.
For octocrylene no SCCS opinion was available, but a paper by Odio *et al.* (1994) described three different toxicity studies where reproductive and developmental effects of octocrylene were investigated. However no significant effects were seen. A 90 day study in rabbits using doses up to 534 mg/kg/day (by topical application) showed no effects on testicular and epididymal morphology,

and no effects on sperm count and motility. A developmental toxicity study in mice, testing doses up to 1000 mg/kg/day from GD8-12 showed no effect on pup size and survival on PND 3, and a developmental toxicity study in rabbits using up to 267 mg/kg/day from GD6-18 showed no effect on implantations, resorptions, number of live or dead foetuses or body weight on GD 29. It was therefore concluded that no reproductive or developmental toxicity was observed at the tested doses of octocrylene (Odio *et al.* 1994).
Since no adverse effects were seen on testicular and epididymal morphology or on sperm quality in the 90-day study, octocrylene does not seem to be a potent antiandrogen. However because of lack of data on mechanistic properties of the compound (i.e. binding to steroid hormone receptors), and lack of screening studies (Uterotrophic or Hershberger) investigating endocrine sensitive endpoints, or even better developmental studies testing endpoints like anogental distance, nipple retention and reproductive organ weights, with the available information it is not possible to conclude whether octocrylene has endocrine disrupting potential or not.

Table 8. UV-filters where more data on reproductive and developmental toxicity exists

INCI-name	CAS no.	Chemical name / INN / XAN /other used names	Max conc. allowed as UV filter in cosmetic products	Open literature	SCCS opinion	Assessment of reproductive toxicity in opinion:
OCTOCRYLENE	6197-30-4	2-Cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester / Octocrilene	10% (as acid)	A 90 day study in rabbits showed no effects on testicular and epididymal morphology, and no effects on sperm count and motility. A developmental toxicity study in mice showed no effect on pup size and survival on PND 3, and a embryotoxicity study in rabbits showed no effect on implantations, resorptions, number of live or dead foetuses or body weight (Odio *et al.* 1994).	no available opinion, substance approved in 1995	-
TITANIUM DIOXIDE	13463-67-7	Titanium dioxide	25%	In the form of nano particles, TiO2 can be found in testes and brains of perinatally exposed 6 week old mice, after subcutaneous administration to the dams. No effect on reproductive organ weights, but abnormal testicular morphology and reduced sperm production was seen (Takeda *et al.* 2009).	Comprehensive opinion from 1998. Additional opinion from 2007 in safety of nano materials.	No reproductive tests reported. Extensive tests for percutaneous absorption, mostly *in vitro*, indicate that absorption does not occur, either with coated or uncoated material. The toxicological profile of this material does not give rise to concern in human use, since the substance is not absorbed through the skin. In view, also, of the lack of percutaneous absorption, a calculation of the margin of safety has not been carried out. In the new opinion it is stated that TiO2 in nano form should be tested in toxicity studies.

For titanium dioxide a SCCS opinion existed in which no reproductive tests were reported. However, it was concluded that this was not a problem, as extensive tests for percutaneous absorption, mostly *in vitro*, indicate that absorption does not occur, either with coated or uncoated material. The toxicological profile of this material does therefore not give rise to concern in human

use, since the substance is not absorbed through the skin. In view, also, of the lack of percutaneous absorption, a calculation of the margin of safety has not been carried out.
Some evidence from the open literature however indicates that in the form of nano particles, TiO2 can cause developmental toxicity in CD-1mice. In dams dosed orally with a single dose during gestation, the TiO2 particles reduced the developmental success of the mice and caused a significant increase in fetal deformities and mortality (Philbrook *et al.* 2011). In another mouse study where TiO2 had been administered subcutaneously to the dams during gestation, the compound could be found in testes and brains of the perinatally exposed mice at 6 weeks of age, and the exposure caused abnormal testicular morphology and reduced sperm production. No effect on reproductive organ weights was seen (Takeda *et al.* 2009).

4.2. Results and discussion on ecotoxicity data

All 23 UV-filters were also evaluated for their potential endocrine disrupting effect on ecosystems, according to the literature search criteria described in the methods section. Relevant ecotoxicological studies were only found for four of the compounds, benzophenone-4, butyl-methoxybenzoylmethan, octocrylene and titanium dioxide. Conclusions on the endocrine disrupting potential of the four UV-filters, and study summaries of the studies which have led to these conclusions are provided below.

Kaiser *et al.* (2012) aimed at evaluating the effects of three UV filters, including **octocrylene** and **butyl-methoxydibenzoylmethane** on the following aquatic organisms: *Chironomus riparius, Lumbriculus variegatus, Melanoides tuberculata, Potamopyrgus antipodarum* (0.08-50 mg/kg sediment dw). Additionally, two direct sediment contact assays utilising zebrafish (*Danio rerio*) embryos and bacteria (*Arthrobacter globiformis*) were conducted (10-1000 mg/kg sediment dw). Endpoints included number of embryos and developmental disorders. Neither **octocrylene** nor **butyl-methoxydibenzoylmethane** showed any effects on any of the tested organisms in the tested concentration ranges.

The third compound for which ecotoxicological data existed was **benzophenone-4**. Kunz *et al.* (2006) investigated whether benzophenone-4 would show estrogenic activity in fathead minnows, by the induction potential of vitellogenin after 14 days of aqueous exposure. The fish were exposed to five different concentrations of benzophenone-4: 11 (median measured), 100 (nominal), 500 (nominal), 1048 (median measured) or 4897 µg/L (median measured). However, no significant differences were found between exposed and control groups.
In 2010 both the acute and the chronic effects of benzophenone-4 were investigated on *Daphnia magna* (Fent *et al.* 2010). In the acute toxicity test (OECD guideline 202), the LC50 value (48 h) was 50 mg/L. The chronic toxicity of benzophenone-4 was determined in a 21 d reproduction study performed according to OECD guideline 211 with exposure concentrations of 0.128-5 mg/L. No adverse effects on either the number or sex of the offspring, or body size were observed after exposure to benzophenone-4.

Zucchi *et al.* (2011) evaluated the effects of benzophenone-4 in eleuthero-embryos and in the liver, testis and brain of adult male fish on the transcriptional level, by focusing on target genes involved in hormonal pathways. Eleuthero-embryos and males of zebrafish were exposed up to 3 days after hatching and for 14 days, respectively, to benzophenone-4 concentrations between 30 and 3000 µg/L. In eleuthero-embryos transcripts of vtg1, vtg3, esr1, esr2b, hsd17ß3, cyp19b cyp19a, hhex and pax8 were induced at 3000 µg/L benzophenone-4, which points to a low estrogenic activity and interference with early thyroid development, respectively. In adult males benzophenone-4 displayed multiple effects on gene expression in different tissues. In the liver vtg1, vtg3, esr1 and esr2b were down-regulated, while in the brain, vtg1, vtg3 and cyp19b transcripts were upregulated. In conclusion, the transcription profile revealed that benzophenone-4 interfered with the expression of genes involved in hormonal pathways and steroidogenesis. The effects of benzophenone-4 differed in early life stages and adult tissues and pointed to estrogenic activity in eleuthero-embryos and in adult brain, and to anti-estrogenic activity in the liver. Taken together, the results from this study indicated that benzophenone-4 does interfere with the sex hormone system of fish.

No ecotoxicity studies on the endocrine disrupting potential of traditional bulk **titanium dioxide** (TiO_2) have been found. However, several ecotoxicological reproduction studies with titanium dioxide nanomaterial exposures exist. These studies have presented varied results. The exposure of *Daphnia magna* to Degussa P25 for 21 days showed a LC50 of 2.62 mg/L and alteration of the reproduction and growth rates (EC50: 0.46 mg/L) (Zhu *et al.*, 2010), while exposure for the same period to different types of BASF nano-TiO_2 did not cause mortality but reduced the reproductive capacity (EC50: 26.6 mg/L) (Wiench *et al.*, 2009). Kim et al. (2010) did not find reproductive impairment but reported a 70% mortality rate in *D. magna* exposed for 21 days to 5 mg/L of Sigma Aldrich nano-TiO_2. Seitz *et al*. (2013) assessed the chronic (21 d) ecotoxicity of two nano-TiO_2 products (A-100; P25) to *D. magna*. A semi-static and a flow-through exposure scenario were compared. Utilizing the semi-static test design, a concentration as low as 0.06 mg/L A-100 significantly reduced the reproduction of daphnia. In contrast, no implication in the number of released offspring was observed during the flow-through experiment with A-100. Likewise, P25 did not adversely affect reproduction irrespective of the test design utilized. The exposure of *D. magna* and *Chironomus riparius* larvae to 1 mg/L Sigma Aldrich nano-TiO_2 for 96 h did not lead to alteration in growth, mortality or reproduction (Lee *et al*., 2009). A 25% inhibition of reproduction of *Ceriodaphnia dubia* was observed after 7 days exposure to 8.5 mg/L nano-TiO_2 (Hall *et al*., 2009). For *Ceriodaphnia affinis*, a statistically significant drop relative to controls was recorded in the mean number of broods and in the mean brood size per female over 7 days after exposure to TiO_2 (from 0.2 mg/L) (Tomilina *et al*., 2011). The decrease in the brood size was due to the longer maturation of organisms rather than a decrease in the number of embryos in the brood. Five days of dietary exposure of juvenile nematode *Caenorhabditis elegans* to nano-TiO_2 reduced the number of eggs inside the worm and offspring per worm (Wang *et al*., 2009). Likewise, 24 h exposure of *Caenorhabditis elegans* to nano-TiO_2 reduced the number of eggs of the worms (Roh *et al*., 2010). McShane *et al.* (2012) found that exposure of earthworms (*Eisenia andrei* and *Eisenia fetida*) to TiO_2 nanomaterials had no significant effect on juvenile survival and growth, adult earthworm survival, cocoon production, cocoon viability, or total number of juveniles hatched from these

cocoons. In abalone (*Haliotis diversicolor supertexta*), no developmental effects of nano-TiO_2 were observed at 2 mg/L but concentrations ≥ 10 mg/L caused hatching inhibition and malformations (Zhu *et al*., 2011). The presence of 2 mg/L nano-TiO_2 increased the toxicity of TBT up to 20-fold compared with TBT alone. Exposure of zebrafish (*D. rerio*) eggs to nano-TiO_2 for 96 h at concentrations of up to 500 mg/L did not cause alterations in the survival and hatching rates, or malformations (Zhu *et al.*, 2008). The inconsistency in the results listed above could be due to variations in nanoparticle size, surface area, surface chemistry, as well as interactions between nanomaterials and ions or dissolved organic matter in complex media.

In conclusion, the ecotoxicity studies did not lead to any conclusions regarding the endocrine disrupting potential of the examined UV-filters. Neither octocrylene nor butyl-methoxydibenzoylmethane showed any effects on any of the tested organisms in the tested concentration ranges. Benzophenone-4 displayed multiple effects on the expression of genes involved in hormonal pathways and steroidogenesis in zebrafish indicating an interference with the sex hormone system of fish. However, in a fathead minnow study no significant effects on vitellogenin levels were seen at the tested concentrations, and furthermore, no adverse effects on either the number or sex of the offspring were observed in daphnia after exposure to benzophenone-4.Ecotoxicological reproduction studies with titanium dioxide nanomaterial exposures have presented varied results, however, no data exist on the compounds endocrine disrupting potential.

4.3. Read Across and QSAR

Based on the present review of the literature, it is very clear that test data on the endocrine disrupting potential of these 23 UV-filters is scarce. Therefore, QSAR and read across to other UV-filters, where more knowledge on endocrine disruption is present, seem very relevant. The open literature was searched for published QSAR predictions regarding endocrine disrupting potential of UV-filters, however none were found. Of the seven UV-filters previously assessed for their endocrine disrupting potential (Hass *et al.*, 2012), four were benzophenones [i.e. benzophenone 1, 2 and 3; dihydroxybenzophenone], two were benzylidene camphor compounds [4-methylbenzylidene camphor (4-MBC); 3-benzylidene camphor (3-BC)] and one was a methoxy cinnamate compound (OMC). Much more information was available regarding the endocrine disrupting potential of these 7 UV-filters than of the presently evaluated 23 UV-filters, and based on the available data, they were all evaluated as either endocrine disrupters in group 1 or suspected endocrine disrupters in group 2a (according to the Danish criteria for EDCs) (Hass *et al.*, 2012). In Appendix 1, the chemical structures of all 30 UV-filters are shown. The four UV-filters in the top row (BP-2, 4-MBC, 3-BC and OMC) were evaluated as endocrine disrupters in group 1, and the three UV-filters in the second row (BP-1, BP-3 and dihydroxy BP) were evaluated as suspected endocrine disrupters in group 2a. The results leading to these conclusions are summarized below the table.

In the previous evaluation BP-2 was evaluated as an endocrine disrupter in group 1 and three other BPs were evaluated as suspected endocrine disrupters and placed in group 2a. This indicates that BP-4 and 5 (the two benzophenones included in the present evaluation), may also have endocrine

disrupting potential. However, there seemed to be differences in both the mode of action and in the potential for adverse effects of the previously evaluated benzophenones and read-across was therefore not done for those benzophenones. Thus, it is uncertain how likely BP-4 and 5 are to also have endocrine disrupting potential, based on a read across.

Two of the previously evaluated UV-filters were benzylidene camphor compounds (4-MBC and 3-BC). This was also the case for two of the presently evaluated UV-filters [benzylidene camphor sulphonic acid & polyacrylamidomethyl benzylidene camphor]. Additionally, two more UV-filters had camphor groups in them [camphor benzalkonium methosulfate & terephthalyidene dicamphor sulfonic acid]. How much the 'camphor' groups is of relevance with regard to endocrine disrupting potential is uncertain, but the fact that both 4-MBC and 3-BC were evaluated as endocrine disrupters in group 1 might indicate that the two presently evaluated benzylidene camphor UV-filters (and possibly also the two compounds which had just camphor groups in their chemical structure), could have endocrine disrupting potential.

The last UV-filter in group 1 in the previous evaluation was ethylhexyl methoxycinnamate (OMC). One compound included in the present assessment [isoamyl p-methoxycinnamate] seemed to have a chemical structure somewhat resembling that of OMC. Whether this chemical resemblance is indicative of isoamyl p-methoxycinnamate also having endocrine disrupting potential, is presently unknown.

The chemical structures of the remaining 16 compounds did not at first glance seem to resemble any of the previously evaluated UV-filters. Performance of QSAR analysis may determine whether their chemical structures resemble other endocrine disrupters, and may also indicate whether any of these 23 chemicals have endocrine disrupting potential. This would increase the knowledge compared to what is known based on the present very scarce knowledge of their endocrine disrupting potential.

5 Summary and conclusions

Summarized in table 9 are the conclusions on endocrine disrupting potential of the 23 examined UV-filters.

Table 9. Overview of the evaluations regarding human health of the 23 UV-filters, based on *in vitro* and animal data, from the SCCS opinions and the open literature

Available information	Group	No. of UV-filters	UV-filter names
No data on teratogenicity No data on endocrine mode of action (MOA) No data on endocrine effect (Table 2)	A	6	Benzophenone-5*; Benzylidene camphor sulfonic acid*; Diethyhexyl butamido triazone; Polyacrylamidomethyl benzylidenecamphor*; Polysilicone-15; Terephtalylidene dicamphorsulfonic acid;
No data on teratogenicity Limited data on endocrine mode of action (MOA) No data on endocrine effect (Table 3)	B	2	Benzophenone-4 *¤ ; Octisalate
No teratogenic effect *in vivo* No data on endocrine MOA No data on endocrine effect (Table 4 & 5)	C	6	Ethylhexyl triazone; Disodium phenyl dibenzimidazole tetrasulfonate; Drometrizole trisiloxane; Peg-25 paba; Diethylamino hydroxybenzoyl hexyl benzoate; Isoamyl p-methoxycinnamate *
No endocrine MOA or not tested No uterotrophic effect (Table 6)	D	4	Camphorbenzalkonium methoslfate; Bisethylhexyloxyphenol methoxyphenyltriazine; Methylene bisbenzotriazolyltetramethylbutylphenol; Phenylbenzimidazole sulfonic acid
Some signs of endocrine MOA No uterotrophic effect (Table 7)	E	3	Butyl methoxydibenzoylmethane ¤; Ethylhexyl dimethyl PABA; Homosalate
Not embryotoxic, not dev. toxic, not repro-toxic in adult male No information on endocrine MOA or endocrine effect (Table 8)	F	1	Octocrylene ¤
Possible reproductive toxicant No information on endocrine MOA or endocrine effect (Table 8)	G	1	Titanium dioxide ¤

¤The UV-filters that have also been evaluated for their ecotoxicological endocrine disrupting potential
*The UV-filters that structurally resemble known or suspected endocrine disrupting UV-filters in a read across assessment, see text for details

For 12 of the compounds (groups A & C), no information on endocrine disrupting potential could be found in either SCCP opinions or in the open literature. For the UV-filters in group A also no

information on teratogenicity was available, and it is therefore impossible to say if these compounds have even been tested for reproductive toxicity in animal studies.

The two compounds placed in group B, have been tested for endocrine disrupting mode of action *in vitro*, whereas no rodent *in vivo* studies have been performed. *In vitro*, benzophenone-4 was shown to have no agonistic or antagonistic effect on the androgen receptor (Ma *et al*. 2003), but was not tested for its affinity to the estrogen receptor. When investigated for its endocrine disrupting potential in ecosystems, it displayed multiple effects on the expression of genes involved in hormonal pathways and steroidogenesis in zebrafish, whereas no significant effects on vitellogenin levels in fathead minnows and no adverse effects on either number or sex of the offspring in daphnia were observed. The other compound placed in group B, octisalate was investigated for estrogenic activity *in vitro* and here the UV-filter showed weak estrogenic activity. For both UV-filters there are therefore some indications that they may potentially act as endocrine disrupters, but the data indicating an endocrine mode of action or endocrine disrupting effect are very weak.

For the UV-filters placed in group D & E, results from uterotrophic tests were available, and for all seven compounds these were negative. For the four compounds placed in group C, either no *in vitro* studies investigating estrogen receptor binding were present, or the results of these tests were negative - whereas for the three compounds in group D, some indications of estrogenic mode of action were present. However, regardless of the UV-filters' abilities to bind to the estrogen receptor, no adverse uterotrophic effects were seen, indicating lack of *in vivo* estrogenic potential for these seven compounds. One of the compounds in group E, butylmethoxydibenzoylmethane, was also investigated for its endocrine disrupting properties in the environment, and here it showed no adverse effect on endpoints like number of embryos or developmental disorders, in any of the tested organisms. The three compounds in group E were also investigated for AR agonism/antagonism *in vitro*, and the results differed somewhat depending on which type of study had been performed. Since no *in vivo* studies investigating the antiandrogenic effects of the compounds have been performed, it is difficult to conclude anything on their endocrine disrupting potential with regard to the possible androgenic/antiandrogenic mode of action.

Octocrylene was placed by itself in group F because some more data were available for this compound regarding reproductive toxicity data. Data on endocrine disrupting potential was however, still scarce. Since no adverse effects on testicular and epididymal morphology or on sperm quality were seen in the 90-day study, octocrylene did not seem to be a potent anti-androgen. However, because of lack of data on mechanistic properties of the compound (i.e. binding to steroid hormone receptors), and lack of screening studies (Uterotrophic or Hershberger) investigating endocrine sensitive endpoints, or even better developmental studies testing endpoints like anogenital distance, nipple retention and reproductive organ weights, it is not possible to conclude whether octocrylene has endocrine disrupting potential or not. In the performed ecotoxicology study, octocrylene showed no adverse effects on endpoints like number of embryos or developmental disorders, in any of the tested organisms.

Finally titanim dioxide was also placed in its own group, because data indicate that when tested as a nano particle, it may act as a reproductive toxicant. However, no data exist on its endocrine disrupting potential.

Some of the 23 UV-filters that have been evaluated in the present report have structural similarities to previously assessed UV-filters, which were evaluated as either suspected endocrine disrupters or as endocrine disrupting chemicals. This was the case for benzophenone 4 and 5, benzylidene camphor sulphonic acid, polyacrylamidomethyl benzylidene camphor and isoamyl p-methoxycinnamate. Whether these structural similarities to known or suspected endocrine disrupters indicate endocrine disrupting potential, is somewhat uncertain.

In conclusion, very little is known on the endocrine disrupting potential of these 23 UV-filters. For 14 of the 23 assessed UV-filters (placed in groups A, B and C in table 9) no *in vivo* studies in rodents, assessing endpoint that are sensitive to endocrine disruption, have been performed, and it was therefore not possible to conclude anything on their endocrine disrupting potential, with regard to human health. Of the 23 compounds, only four were tested for their ecotoxicological potential. Two of these [octocrylene and butyl methoxydibenzoylmethane] showed no adverse effects in the used test systems. Titanium dioxide was tested as a nanomaterial in a large number of ecotoxicological reproduction studies, however yielding quite varied results and no answers as to its endocrine disrupting potential. Results for benzophenone-4 showed multiple effects on gene expression in steroidogenesis in zebrafish, but no effects on vitellogenin levels in fathead minnows or on the number or sex of the offspring in daphnia. Benzophenone-4 also showed no affinity to the androgen receptor *in vitro*.
Seven of the UV-filters (placed in groups C & D) were tested in the Uterotrophic assay, and regardless of their estrogenic potential *in vitro,* none of them caused increased uterine weights, indicating lack of estrogenic potential *in vivo*. The three compounds in group E were also investigated for AR agonism/antagonism *in vitro*, and the results differed somewhat depending on which type of study had been performed. However, since no *in vivo* studies investigating the antiandrogenic effects of the compounds were present, it is difficult to conclude anything on their endocrine disrupting potential with regard to the possible androgenic/antiandrogenic mode of action. Information on human health endocrine disrupting potential of last two UV-filters [octocrylene and titanium dioxide] was also scarce. Since no adverse effects on testicular and epididymal morphology or on sperm quality were seen in a 90-day study of octocrylene, this UV-filter did not seem to be a potent anti-androgen. Titanium dioxide, tested as a nano-particle, was the only investigated compound which showed signs of being a developmental and reproductive toxicant, as it caused a significant increase in fetal deformities and mortality in one study, and abnormal testicular morphology and reduced sperm production in another. However, whether these adverse effects were mediated by endocrine disruption has not been investigated. Read across assessment showed possible resemblance of the chemical structures of some of the presently evaluated UV-filters to known or suspected endocrine disrupting UV-filters, however more knowledge on the endocrine disrupting potential of the presently evaluated UV-filters could be

obtained by doing QSAR analyses. Unfortunately no published reports of such analysis were present in the open literature.

6. References

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CAMPHOR BENZALKONIUM METHOSULFATE
SCCP/1015/06: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_080.pdf

HOMOSALATE
SCCP/1086/07: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_097.pdf

PHENYLBENZIMIDAZOLE SULFONIC ACID
SCCP/1056/06: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_079.pdf

POLYSILICONE-15
SCCS/1246/10: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_024.pdf

TITANIUM DIOXID
SCCNFP/0005/98: http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out135_en.pdf
and new opinion on the safety of nanomaterials in cosmetic products
SCCP/1147/07: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf

DIETHYLAMINO HYDROXYBENZOYL HEXYL BENZOATE
SCCP/1166/08: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_130.pdf

PEG-25 PABA
Short opinion from 1997, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out05_en.htm

ISOAMYL P-METHOXYCINNAMATE
Short opinion from 1997, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out06_en.htm

ETHYLHEXYL TRIAZONE
Short opinion from 1996, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out04_en.htm

DROMETRIZOLE TRISILOXANE
Short opinion from 1997, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out03_en.htm

DIETHYLHEXYL BUTAMIDO TRIAZONE
Short opinion from 1997, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out29_en.htm

ETHYLHEXYL SALICYLATE
Short opinion from 1995, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out26_en.htm

ETHYLHEXYL DIMETHYL PABA
Short opinion from 1999, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out54_en.htm

BENZOPHENONE 4 og BENZOPHENONE 5
Short opinion from 1999, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out57_en.htm

METHYLENE BIS-BENZOTRIAZOLYL TETRAMETHYLBUTYLPHENOL
Short opinion from 1999, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out55_en.htm

DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE
Short opinion from 1999, no references:
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out53_en.htm

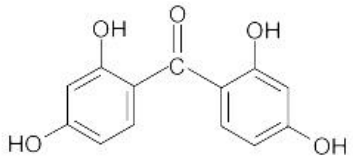
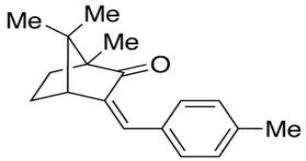
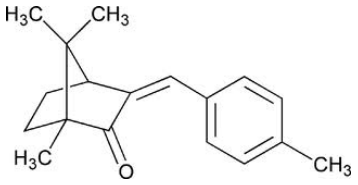
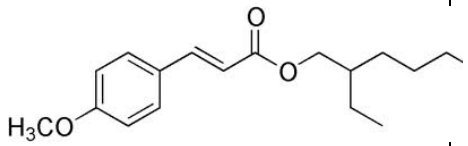
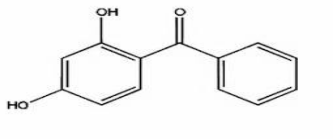
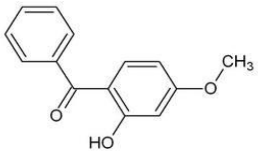
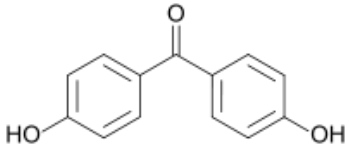
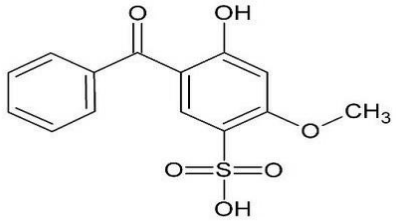
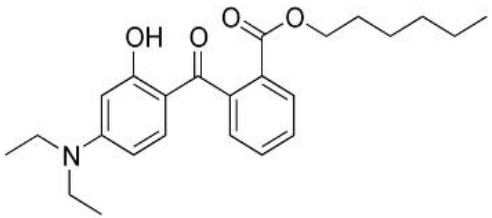
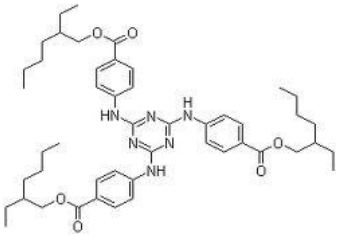
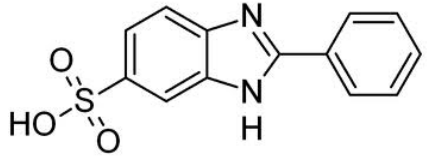
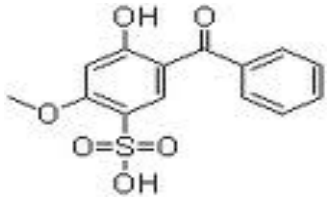
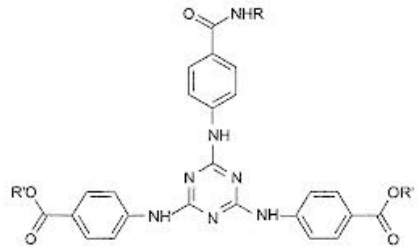
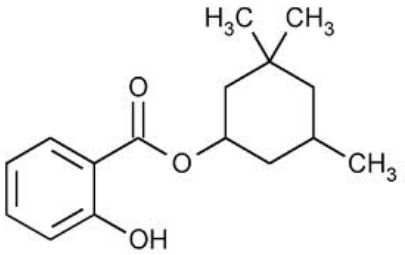
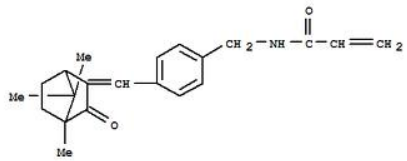
BIS ETHYLHEXYLOXYPHENOL METHOXYPHENOL TRIAZINE
Short opinion from 1998, no references:
http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out52_en.pdf.

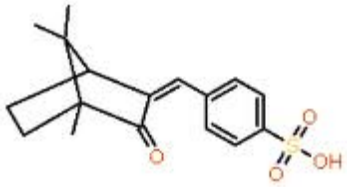
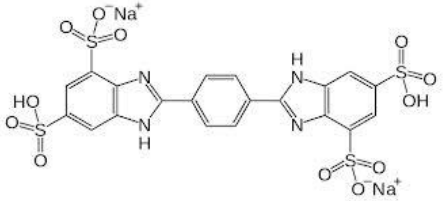
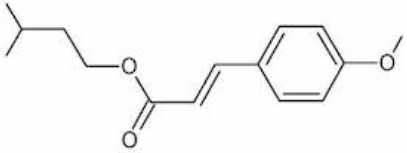
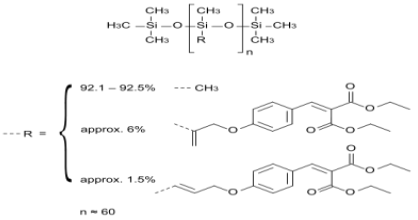
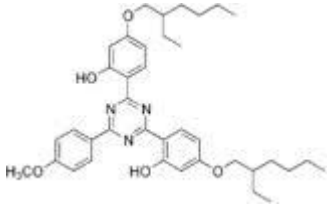
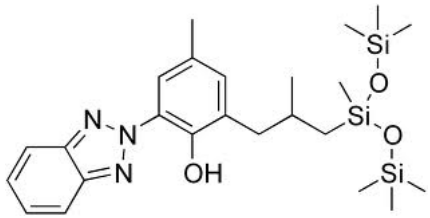
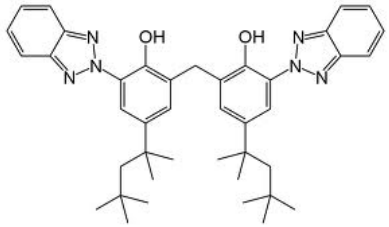
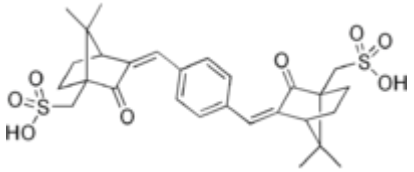
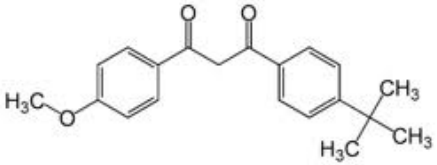
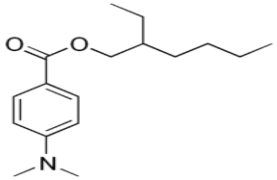
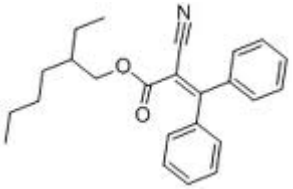
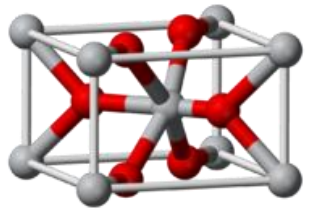
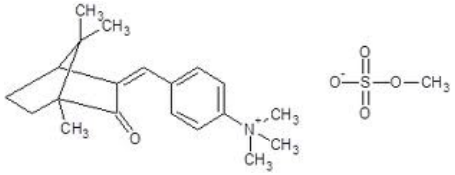
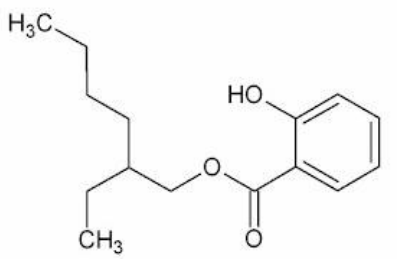
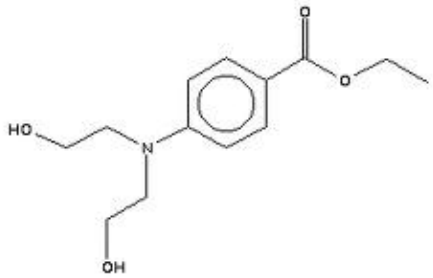
7. List of abbreviations

AR: androgen receptor
3-BC: 3-benzylidene camphor
BP: benzophenone (BP1, 2, 3, 4 &5)
CEHOS: Danish Centre on Endocrine Disrupters
Cyp 19a: aromatase
DTU: Technical University of Denmark (National Food Institute)
E2: estradiol
EDCs: endocrine disrupting chemicals
ER: estrogen receptor
GD: gestation day
hER: human estrogen receptor
INCI-name: international nomenclature of cosmetic ingredients
LC50: lethal concentration 50%
MCF7: breast cancer cell line. Michigan Cancer Foundation, institute where the cell line was established.
4-MBC: 4-metylbenzylidene camphor
NOAEL: no adverse effect level
OMC: ethylhexyl methoxycinnamate
PND: postnatal day
R1881: metribolone or methyltrienolone (a potent androgen)
SCCS: Scientific Committee on Consumer Safety
SDU: University of Southern Denmark (Institute of Biology)
TBT: tributyltin
TiO2: titanium dioxide
UV: ultra violet
Vtg: vitellogenin
QSAR: quantitative structure–activity relationship

Appendix 1.

Chemical structure of the 7 UV-filters evaluated in Hass *et al.* (2012) for their endocrine disrupting properties, and of the 23 UV-filters evaluated in the present report. The four UV-filters in the top row were evaluated as endocrine disrupters in group 1, and the three UV-filters in the second row were evaluated as suspected endocrine disrupters in group 2a, based on the Danish criteria for endocrine disrupters (Hass *et al*. 2012). Results leading to these conclusions are summarized below the table for all seven of the UV-filters.

BENZOPHENONE-2 	4-METHYLBENZYLIDENE CAMPHOR 	3-BENZYLIDENE CAMPHOR 	ETHYLHEXYL METHOXYCINNAMATE 
BENZOPHENONE-1 	BENZOPHENONE-3 	DIHYDROXYBENZOPHENONE 	
BENZOPHENONE-4 	DIETHYLAMINO HYDROXYBENZOYL HEXYL BENZOATE 	ETHYLHEXYL TRIAZONE 	PHENYLBENZIMIDAZOLE SULFONIC ACID 
BENZOPHENONE-5 	DIETHYLHEXYL BUTAMIDO TRIAZONE 	HOMOSALATE 	POLYACRYLAMIDOMETHYL BENZYLIDENE CAMPHOR 

BENZYLIDENE CAMPHOR SULFONIC ACID 	DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE 	ISOAMYL P-METHOXYCINNAMATE 	POLYSILICONE-15 
BIS-ETHYLHEXYLOXYPHENOL METHOXYPHENYL TRIAZINE 	DROMETRIZOLE TRISILOXANE 	METHYLENE BIS-BENZOTRIAZOLYL TETRAMETHYLBUTYLPHENOL 	TEREPHTHALYLIDENE DICAMPHOR SULFONIC ACID 
BUTYL METHOXYDIBENZOYL METHANE 	ETHYLHEXYL DIMETHYL PABA 	OCTOCRYLENE 	TITANIUM DIOXIDE 
CAMPHOR BENZALKONIUM METHOSULFATE 	ETHYLHEXYL SALICYLATE 	PEG-25 PABA 	

Benzophenone-2 (BP-2), CAS 131-55-5
There is strong evidence that BP-2 has estrogenic and possibly also an anti-androgenic mode of action *in vitro*. BP2 can also affect the thyroid system *in vitro*, by inhibiting the enzyme thyroid peroxidase (TPO), by binding to the thyroid receptor and by affecting thyroid hormone signaling in the testes. There is furthermore strong evidence that BP-2 increases uterus weight in the Uterotrophic assay, showing estrogenic activity *in vivo*. Only one developmental study of BP-2 has been described in the open literature. It showed significantly increased incidence of hypospadias in male mouse foetuses. No effect on anogenital distance in the male offspring was seen, indicating that the adverse effect was not mediated by an anti-androgenic mechanism. BP2 can also affect the thyroid hormone system *in vivo*, as shown by reduced thyroid hormone levels in adult rats from several studies. BP-2 induces vitellogenin in fish. One study showed significant estrogenic effects of BP-2 on vitellogenin induction, secondary sex characteristics, gonadal development, and reproduction in fish. The induction of vitellogenin demonstrates an estrogenic mode of action. Cessation of spawning (and thereby reproduction) is an adverse apical effect. It has been shown that benzophenone-2 decreases intrafollicular T4-content in fish which classifies BP-2 as a thyroid gland function disruptor in fish.
Evaluation: ED in Category 1.

4-methylbenzylidene camphor (4-MBC), CAS 36861-47-9
In vitro, there is strong evidence of estrogenic activity, as 4-MBC has been shown to bind to the ER, alter gene transcription and cause proliferation of MCF-7 cells. No androgenic or anti-androgenic effects *in vitro* were seen in one study, while anti-androgenic activity and strong progesterone activity was seen in another. 4-MBC can also affect the thyroid system *in vitro*, by binding to the thyroid receptor. The evidence of estrogenic activity from short term *in vivo* studies is conflicting, however increases in uterine weights and histopathological effects in uterus and vagina have been observed after longer exposure scenarios. Furthermore a large number of endocrine sensitive endpoints such as reproductive organ weights, timing of sexual maturation, impaired sexual behaviour have been shown to be affected in the developmental studies. Also, changes in LH, FSH and GnRH levels have been observed. In fish, 4-methylbenzylidene camphor at high concentrations induces estrogen-responsive gene products including vitellogenin.
Evaluation: ED in Category 1.

3-benzylidene camphor (3-BC), CAS 15087-24-8
Only few *in vitro* studies have been performed with 3-BC, however these show some evidence of endocrine disrupting modes of action, especially estrogenic mode of action.The available *in vivo* studies show strong evidence of estrogenic effects. In a screening study for estrogenic effect, 3-BC has been shown to increase uterine weight in immature rats, and in reproductive studies, perinatal 3-BC exposure has been shown to cause delayed sexual maturation, decreased relative epididymis and seminal vesicle weights in adult male offspring, while female offspring showed irregular oestrous cyclicity and strongly impaired sexual behaviour. In fish, 3-BC has been shown to induce vitellogenin and cause significant effects on reproduction.
Evaluation: ED in Category 1.

Ethylhexyl methoxycinnamate (OMC), CAS 5466-77-3
Some *in vitro* studies of OMC have shown binding to the estrogen receptor, while others have not, resulting in conflicting evidence on estrogenic mode of action. Other modes of action such as binding to the thyroid and progesterone receptor *in vitro* have also been seen. There is strong evidence that OMC can affect the endocrine system *in vivo*. Slight but significant increases in uterine weights have been seen in both intact immature and adult ovarietectomized rats. In a 2-generation study, a significant decrease in sperm cell number was seen, while another reproductive study has shown developmental OMC exposure to cause several adverse reproductive effects in the offspring, including reduced reproductive organ weights, reduced reproductive hormone levels, reduced sperm counts and neurobehavioural effects. OMC can also interfere with the hypothalamo-pituitary-thyroid (HPT) axis *in vivo*, as a number of studies have shown reduced levels of thyroxine in the blood.OMC affects the transcription of genes involved in hormonal pathways including vitellogenin in most fish studies.
Evaluation: Endocrine disrupter in Category 1.

4,4´-dihydroxybenzophenone, CAS 611-99-4
The *in vitro* data show strong evidence that 4,4'-dihydroxybenzophenone has estrogenic and possibly also anti-androgenic mode of action. Only one *in vivo* study investigating endocrine disruption has been performed. It showed a significant increase in uterine weight in an Uterotrophic assay using immature rats, showing that an estrogenic effect is also be present *in vivo.* 4,4'-dihydroxybenzophenone did not result in significant vitellogenin induction in the fish.
Evaluation: Suspected ED in Category 2a.

Benzophenone-1 (BP-1), CAS 131-56-6
In vitro results show strong evidence that BP-1 has an estrogenic mode of action, while *in vitro* data for anti-androgenicity are conflicting. BP-1 has been shown to increase uterine weight in several Uterothrophic assays in rats, showing an estrogenic effect *in vivo*. No developmental toxicity studies with Benzophenone-1 have been found. Two ecotoxicology studies have shown that benzophenone-1 induces vitellogenin in fish.
Evaluation: Suspected ED in Category 2a.

Benzophenone-3 (BP-3), CAS 131-57-7
A large number of *in vitro* studies with BP-3 have been performed. Many of them show estrogenic modes of action, while this mode of action is not seen in others. Antagonism of the androgen receptor and the progesterone receptor has also been shown and BP-3 has also been shown to affect the thyroid system *in vitro*, by binding to the thyroid receptor. *In vivo* there is only limited evidence of estrogenic activity. Only one study has shown increased uterine weight in the Uterotrophic assay, whereas other studies have not found this, however, all these later studies tested doses below the LOAEL for the uterotrophic effect. Benzophenone-3 induces vitellogenin in fish in one study but not in two other studies. The study showing a response on vitellogenin also shows reduced percentage of hatching of fish eggs.
Evaluation: Suspected ED in Category 2a.