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REVIEW ARTICLE

Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters

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Summary

Today, topical application of sunscreens, containing ultraviolet-filters (UV-filters), is preferred protection against adverse effects of ultraviolet radiation. Evidently, use of sunscreens is effective in prevention of sunburns in various models. However, evidence for their protective effects against melanoma skin cancer is less conclusive. Three important observations prompted us to review the animal data and human studies on possible side effects of selected chemical UV-filters in cosmetics. (1) the utilizationof sunscreens with UV-filters is increasing worldwide; (2) the incidence of the malignant disorder for which sunscreens should protect, malignant melanoma, is rapidly increasing and (3) an increasing number of experimental studies indicating that several UV-filters might have endocrine disruptive effects. The selected UV-filters we review in this article are benzophenone-3 (BP-3), 3-benzylidene camphor (3-BC), 3-(4-methyl-benzylidene) camphor (4-MBC), 2-ethylhexyl 4-methoxy cinnamate (OMC), Homosalate (HMS), 2-ethylhexyl 4-dimethylaminobenzoate (OD-PABA) and 4-aminobenzoic acid (PABA). The potential adverse effects induced by UV-filters in experimental animals include reproductive/developmental toxicity and disturbance of hypothalamic–pituitary–thyroid axis (HPT). Few human studies have investigated potential side effects of UV-filters, although human exposure is high as UV-filters in sunscreens are rapidly absorbed from the skin. One of the UV-filters, BP-3, has been found in 96% of urine samples in the US and in 85% of Swiss breast milk samples. It seems pertinent to evaluate whether exposure to UV-filters contribute to possible adverse effects on the developing organs of foetuses and children.

Abbreviations

3-BC, 3-Benzylidene champhor; 4-MBC, 3-(4-Methyl-benzylidene)-camphor; ↑, Increased; ↓, Decreased; AhR, Aryl hydrocarbon receptor; AR, Androgen receptor; BP-3, Benzophenone 3; Bw, Body weight; C3, complement protein 3; Dio1, 5'deiodinase type I; EDC, endocrine disrupting chemicals; ER, oestrogen receptor; ERR1, oestrogen receptor related receptor 1; F0, Parent rats; F1, 1. Generation of offspring; F, female; FDA, Food and Drug Administration; FRTL-5, normal, non-transformed rat thyrocytes; FT3, free triiodothyronine; FT4, free thyroxine; HepG2, Human hepatocarcinoma cell line; hER, human oestrogen receptor; HMS, Homosalat; HPT, axis, Hypothalamic-pituitary-thyroid axis; IGF-I, insulin-like growth factor-I; LOAEL, Lowest observed adverse effect levels; M, male; MCF7, Human breast cancer cells; ME, Malic enzyme; MM, Malignant melanoma; MPO, Medial Preoptic area; N-Cor, Nuclear receptor corepressor; NHANES, National Health and Nutrition Examination Survey; NOAEL, no observed adverse effect levels; OCT, 2-cyano-3,3-diphenyl acrylic acid; OD-PABA, 2-Ethylhexyl 4-dimethylaminobenzoate; OMC, 2-ethylhexyl-4-methoxy cinnamate; ORG2058, PR agonist; PABA, 4-Aminobenzoic acid; PN, Post natal day; PN1, day of birth; PR, progesterone receptor; rtER, rainbow trout oestrogen receptor; SRC-1, steroid receptor coactivator-1; T3, total triiodothyronine ; T4, total thyroxine ; TBG, thyroxine-binding globulin ; TPO, Thyroid peroxidase ; TSH, thyroid-stimulating hormone; U2-OS, cells, human osteosarcoma cells; UVA, Ultraviolet radiation with wavelength A, 320-400 nm; UVB, Ultraviolet radiation with wavelength B, 290-320 nm; VMH, ventromedial hypothalamic nucleus- plays important role in sexual behaviour and receptivity of female rats (191); VTG, vitellogenin (oestrogen-responsive gene products in fish).

Introduction

The first commercial sunscreen was developed in the 1930s to abrogate ultraviolet-B waveband (UV-B), and thus prevent sunburn (Rebut, 1990). In 1970, sunscreens were developed further to protect against both ultraviolet-A waveband (UV-A) and UV-B (Deep, 2010), because of their suggested causal role in the development of skin cancer, in particular malignant melanoma (MM) (Wang *et al.*, 2001; Gandini *et al.*, 2005). Today it is still questionable whether this aim has been achieved. There is no doubt that sunscreens protect against sunburn, solar keratosis, and non-melanoma skin cancer (Thompson *et al.*, 1993; Green *et al.*, 1999; Dupuy *et al.*, 2005). However, the only randomized trial examining the risk of MM after regular sunscreen use, found borderline statistical significance for a reduced incidence of new primary melanoma (Green *et al.*, 2011). In addition, despite use of sunscreens with UV-filters over decades (Figure S1), the incidence of MM is still increasing rapidly (Fig. 1) (Handel & Ramagopalan, 2010). Furthermore, an increasing number of experimental animal and in vitro studies indicated that some UV-filters might have adverse effects as endocrine disrupters.

In light of the high incidence of MM and considering the facts that the protective effect of sunscreens against MM has not been fully proven, we have reviewed the literature on possible endocrine disrupting effects of the most common chemical UV-filters used in cosmetics. Those are benzophenone-3 (BP-3), 3-benzylidene camphor (3-BC), 3-(4-methyl-benzylidene) camphor (4-MBC), 2-ethylhexyl 4-methoxy cinnamate (OMC), Homosalate (HMS), 2-ethylhexyl 4-dimethylaminobenzoate (OD-PABA) and 4-aminobenzoic acid (PABA).

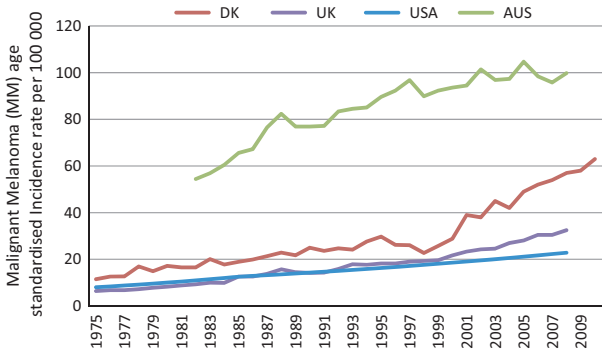

Figure 1 Age standardized MM incidence in USA, AU, NZ, UK and DK (Local cancer statistics). DK: Denmark: 1975–2000 (IARC); 2001–2010: Danish Cancer registers (Sundhedsstyrrelsen, 2012). UK: United Kingdom: (Cancer Research UK). USA: (SEER). AUS: Australia: (Australian Institute of Health and Welfare [AIHW]).

In vitro and in vivo adverse effects of UV-filters

A wide range of in vitro and in vivo studies have identified several UV-filters as endocrine disrupting chemicals (EDC) (European Commission) (Table S1). Table S1 lists results from in vivo and in vitro studies where possible adverse effects of the most common chemical UV-filters in cosmetics (Table 1) were examined. In the following the main results from Table S1 will be summarized. A simplified version of Table S1 is presented in Table 2.

Influence of oestrogenic signalling

In vitro studies, investigating oestrogenic activity of UV-filters, varied in their design and endpoints, which may explain the diverging results. The majority of the in vitro studies reported that BP-3, 4-MBC, OMC, HMC and OD-PABA all exhibit oestrogenic activity (Schlumpf

Table 1 Most common UV-filters in cosmetics

	Genetic name	Product name	Max concentration (%)	Spectrum of action	Approved
Chemical UV-filters	Benzophenon-3	BP-3	6[b]–10[a,c]	UV-A, UV-B	EU, US, AU
	2-cyano-3,3-diphenyl acrylic acid	OCT	10	UV-B	EU, US, AU
	3-Benzylidene camphor	3-BC	2	UV-B	EU
	3-(4-Methyl-benzylidene) camphor	4-MBC	4	UV-B	EU, AU
	2-Ethylhexyl 4-methoxy cinnamate	OMC	7.5[b]–10[a,c]	UV-B	EU, US, AU
	Homosalate	HMS	10[a]–15[b,c]	UV-B	EU, US, AU
	2-Ethylhexyl 4-dimethylaminobenzoate	OD-PABA	8	UV-B	EU, US, AU
	4-Aminobenzoic acid	PABA	5–15[b,c]	UV-B	US, AU
Physical UV-filter	Titanium dioxide		25	Physical	EU, US, AU
	Zinc oxide		25-no limit	Physical	US, AU

[a] List of permitted UV-filters in the Council Directive of the European Committee.
[b] List of permitted UV-filters in the US Food and Drug Administration monograph.
[c] List of permitted UV-filters in the Australian regulatory guidelines for over-the-counter medicines (ARGOM), by the therapeutic Goods Administration.

et al., 2001b, 2004a; Schreurs *et al.*, 2002, 2005; Gomez *et al.*, 2005; Kunz *et al.*, 2006). However, not all of the UV-filters exhibiting in vitro oestrogenic activity were

Table 2 In vitro and In vivo effects of the most common UV-filters, used in cosmetics (increased: ↑; decreased: ↓)

Endpoint	References
BP-3	
Oestrogen activity: In vitro	
Binding to hERα	Morohoshi *et al.* (2005)
↑ Transactivation: ERα, ERβ	Gomez *et al.* (2005), Kunz *et al.* (2006), Kunz & Fent (2006b), Morohoshi *et al.* (2005) and Schreurs *et al.* (2002)
Activation hERβ > hERα	Schreurs *et al.* (2005)
No Antagonism of ERβ transactivation	Schreurs *et al.* (2002)
Antagonistic action > agonistic action on hERα	Kunz & Fent (2006b)
↑ MCF-7 cell proliferation	Schlumpf *et al.* (2001b)
Oestrogen activity: acute in vivo models	
Uterotrophic effect in immature rats	Schlumpf *et al.* (2001b)
Uterus of adult oophorectomized rats: unchanged weight, ↓ ERβ, unchanged ERα, ERR1 and AhR expression	Schlecht *et al.* (2004)
No effect on VTG induction in juvenile fathead minnows	Kunz *et al.* (2006)
Androgen activity: In vitro	
Antagonism of hAR transactivation. No agonistic action	Kunz & Fent (2006b), Ma *et al.* (2003) and Schreurs *et al.* (2005)
Progesterone activity: In vitro	
Antagonism of hPR transactivation	Schreurs *et al.* (2005)
Reproductive organs: long-term exposure of adult animals	
Oral exposure : ↓Epididymal sperm density (rat, mice), ↑Abnormal spermatozoa (mice). Dermal exposure (mice): ↓Epididymal sperm density Dermal exposure (rats): No effect	French (1992)
Oral exposure (mice); ↑Oestrous cycle length, Dermal exposure (rats): No effect	French (1992)
Reproductive organs: developmental toxicity	
No data available	
Thyroid axis	
In vitro: ↑ TR transcription	Schmutzler *et al.* (2007b)
↓ ERR1, unchanged ERα, ERβ and AhR expression in adult oophorectomized rats (5 day)	Schlecht *et al.* (2004)
Additional organ toxicities and general toxicity	
↓Food consumption (2 weeks), ↓Body weight, ↑ Liver weight (13 weeks)	French (1992)
BP-2	
Thyroid axis	
In vitro: ↑hTPO activity Unaltered iodide uptake	Schmutzler *et al.* (2007a)
adult oophorectomized rats (5 days): ↑TSH, ↓T4 Altered Doi-1 activity (liver) Unaltered TPO activity	
3-BC	
Oestrogen activity: In vitro	
Binding to hERβ No binding to hERα	Schlumpf *et al.* (2004a)
↑ transactivation: ERα	Kunz *et al.* (2006) and Kunz & Fent (2006b)
Activation hERβ > hERα	Schreurs *et al.* (2005)
Antagonistic action > agonistic action on hERα	Kunz & Fent (2006b)
↑ MCF-7 cell proliferation	Schlumpf *et al.* (2001b)
Oestrogen activity: Acute in vivo models	
Uterotrophic effect in immature rats (3 days)	Schlumpf *et al.* (2001b)
↑ VTG induction in juvenile rainbow trouts and juvenile fathead minnows	Holbech *et al.* (2002) and Kunz *et al.* (2006)
Androgen activity: In vitro	
No antagonism of hAR transactivation	Ma *et al.* (2003)
Antagonism of hAR transactivation. No agonistic action	Holbech *et al.* (2002), Kunz & Fent (2006b) and Schreurs *et al.* (2005)
Progesterone activity: In vitro	
Antagonism of hPR transactivation	Schreurs *et al.* (2005)
Reproductive organs: Developmental toxicity	
Delayed male puberty F1 rats	Faass *et al.* (2009) and Schlumpf *et al.* (2004b)
Oestrous cycle changes in F1	
↓ weight of uterus (high dose) and prostate (low dose) F1	
Altered gene expression in uterus and prostate F1	
Central nervous system: Developmental toxicity	
Impaired female sexual behaviour in F1 rats	Faass *et al.* (2009) and Schlumpf *et al.* (2004b)
Additional organ toxicities and general toxicity	
Body weight adult F1 (highest dose) (developmental study)	Schlumpf *et al.* (2004b)
↓ length and weight, juvenile fathead minnows (14 days)	Kunz *et al.* (2006)
HMC	
Oestrogen activity: In vitro	
↑ transactivation: hERα,	Gomez *et al.* (2005)
↑ transactivation: hERα > hERβ	Schreurs *et al.* (2002, 2005)

Table 2 *Continued*

Endpoint	References
HMC	
No agonistic action hERα, rtERα	Kunz *et al.* (2006) and Kunz & Fent (2006b)
Antagonism of hERα transactivation,	Kunz & Fent (2006b)
No antagonism at hERα or hERβ	Schreurs *et al.* (2002)
↑ MCF-7 cell proliferation	Schlumpf *et al.* (2001b)
Oestrogen activity: Acute in vivo models	
No uterotrophic effect in immature rats	Schlumpf *et al.* (2001b)
Androgen activity: In vitro	
Antagonism of hAR transactivation. No agonistic action	Ma *et al.* (2003) and Schreurs *et al.* (2005)
Agonistic and antagonistic action on hAR transactivation	Kunz & Fent (2006b)
Progesterone activity: In vitro	
Antagonism of hPR transactivation	Schreurs *et al.* (2005)
4-MBC	
Oestrogen activity: In vitro	
Binding to cytosolic ER	Tinwell *et al.* (2002)
Binding to hERβ	andSchlumpf *et al.* (2004a)
No binding to hERα	Morohoshi *et al.* (2005)
↑ transactivation: hERα, hERβ;	Gomez *et al.* (2005) and Schreurs *et al.* (2002)
Activation hERα > hERβ	Schreurs *et al.* (2005)
Activation hERα < hERβ.	Mueller *et al.* (2003)
No transactivation: hERα, rtERα	Kunz *et al.* (2006), Kunz & Fent (2006b) and Morohoshi *et al.* (2005)
Antagonism of hER	Kunz & Fent (2006b) and Mueller *et al.* (2003)
No antagonism of hER	Morohoshi *et al.* (2005) and Schreurs *et al.* (2002)
↑ ER-mediated MCF-7 cell proliferation	Schlumpf *et al.* (2001b, 2004a) and Tinwell *et al.* (2002)
Oestrogen activity: Acute in vivo models	
Uterotrophic effect in immature rats	Schlumpf *et al.* (2001b) and Tinwell *et al.* (2002)
Uterotrophic effect in immature hairless Nu rats after (dermal exposure)	Schlumpf *et al.* (2001b)
VTG Induction in juvenile fathead minnows : no effect	Kunz *et al.* (2006)
No effect in transgenic juvenile zebra fish	Schreurs *et al.* (2002)
Androgen activity: In vitro	
Antagonism of hAR transactivation. No agonistic action	Kunz & Fent (2006b) and Ma *et al.* (2003)
No antagonism of hAR transactivation	Schreurs *et al.* (2005)

Table 2 *Continued*

Endpoint	References
4-MBC	
Progesterone activity: In vitro	
Antagonism of hPR transactivation	Schreurs *et al.* (2005)
Reproductive organs: Developmental toxicity	
delayed puberty in males (preputial separation)	Durrer *et al.* (2005, 2007) and Hofkamp *et al.* (2008)
↑ prostate duct formation in F1 neonate	
↓ prostate weight, adult F1	
↑ testis weight adult F1	
↑ uterine weight	
Altered expression and sensitivity of oestrogen target genes and coactivators in prostate and uterus	Durrer *et al.* (2005, 2007) and Faass *et al.* (2009)
No effect on onset of female puberty or oestrous cycle	
Reproductive organs: Long-term exposure of adult animals	
Uterus and vagina of adult oophorectomized rats (Oral exposure):	Seidlova-Wuttke *et al.* (2006)
↑ uterus weight, ↑ epithelial/ endometrial thickness,	
unchanged ER, PR and IGF-1expression	
Central nervous system: Developmental toxicity	
Impaired female sexual behaviour in adult F1	Faass *et al.* (2009), Maerkel *et al.* (2005, 2007)
Altered expression and sensitivity of oestrogen target genes in sexually dimorphic brain regions	
Thyroid axis	
in vitro:	Schmutzler *et al.* (2007b)
↓ Iodide uptake	
Developmental study, rats: ↑ thyroid weight in F1, both sexes	Maerkel *et al.* (2007)
↑ TSH and ↑ T3 in female F1	
adult oophorectomized rats (12 weeks):	Schmutzler *et al.* (2007b)
↑TSH, ↓T4, ↑ T3,	
↓ Doi 1 activity (kidney)	
ME activity unchanged (liver, kidney)	
Additional organ toxicities and general toxicity	
developmental study, rats: No effect on body weight in adult F1	Durrer *et al.* (2005, 2007) and Maerkel *et al.* (2007)
developmental study, rats: ↓ thymus weight, adult female F1	Schlumpf *et al.* (2004b)
adult oophorectomized rats (3 month): ↑ bone density	Seidlova-Wuttke *et al.* (2006)
↑ VTG induction and ER α gene expression in liver of male medaka (7 days)	Inui *et al.* (2003)

Table 2 *Continued*

Endpoint	References
OMC	
Oestrogen activity: In vitro	
No binding to hERα	Morohoshi *et al.* (2005)
↑ transactivation: hERα	Gomez *et al.* (2005) and Schreurs *et al.* (2002)
No transactivation: hERα, hERβ, rtERα	Kunz *et al.* (2006), Kunz & Fent (2006b), Morohoshi *et al.* (2005) and Schreurs *et al.* (2002)
Antagonism of hERα transactivation	Kunz & Fent (2006b) and Morohoshi *et al.* (2005)
↑ MCF-7 cell proliferation	Schlumpf *et al.* (2001b)
Oestrogen activity: Acute in vivo models	
Uterotrophic effect in immature rats	Schlumpf *et al.* (2001b)
Uterus of adult oophorectomized rats: ↑weight, ↑ ERβ and C3 expression	Klammer *et al.* (2005)
↑VTG induction in male medaka	Inui *et al.* (2003)
VTG induction in juvenile fathead minnows: no effect	Kunz *et al.* (2006)
Androgen activity: In vitro	
Antagonism > agonism of hAR transactivation	Kunz & Fent (2006b)
No effect on hAR transactivation	Ma *et al.* (2003) and Schreurs *et al.* (2005)
Progesterone activity: In vitro	
Antagonism of hPR transactivation	Schreurs *et al.* (2005)
Reproductive organs: Developmental toxicity	
No effect on puberty in rats	Axelstad *et al.* (2011)
↓ P-testosterone plasma in F1 on PND 16	
↓prostate weight PND 16, adult F1	
altered prostate histology PND 16, adult F1	
↓testis weight PND 16, unchanged in adult F1	
↓ epididymal sperm count adult F1	
No effect on uterus or ovary weight	
Reproductive organs: Long-term exposure of adult animals	
Adult oophorectomized rats (oral exposure):	Seidlova-Wuttke *et al.* (2006)
uterine weight unchanged or slightly ↑	
↑ thickness of uterus epithelium, endometrium and myometrium, and of vagina epithelium	
PR and IGF-1 expression, uterus and vagina	

Table 2 *Continued*

Endpoint	References
OMC	
Thyroid axis	
In vitro:	Schmutzler *et al.* (2007b)
↓ iodide uptake	
↑ TR transactivation (high concentrations)	
of adult oophorectomized rats (5 days):	Klammer *et al.* (2007)
↓ TSH, ↓ T4, ↓ T3	
↑ TSH receptor protein	
TRH expression in hypothalamus unchanged	
↓Doi 1 activity (liver)	
adult oophorectomized rats (3 month):	Schmutzler *et al.* (2004, 2007b)
↓ T4; T3 and TSH unchanged	
↓ Dio1 activity (liver, kidney)	
↑ malic enzyme activity (kidney, T3 target)	
Developmental study, rats (gavage):	Axelstad *et al.* (2011)
Thyroid weight in F1 ↑ PND 16, unchanged in adult F1	
T4 ↓ in male F1 PND 16 and in dams, unchanged in female F1 PND 16 and in adult F1.	
Central nervous system: Developmental toxicity	
Developmental study, rats (gavage):	Axelstad *et al.* (2011)
↓ motor activity in adult female F1	
↑ spatial learning in adult male F1	
Additional organ toxicities and general toxicity	
Developmental oral exposure, 2 generations:	Schneider *et al.* (2005)
↓ body weight gain and ↓ adult body weight in F1 male and female rats (high dose),	
↑ liver weight in female F1	
Developmental study, rats (gavage):	Axelstad *et al.* (2011)
↓ birth weight and body weight gain, body weight of adult F1: males ↓, females normalized	
Adult oophorectomized rats (5 days):	Klammer *et al.* (2005)
↓ serum cholesterol, ↓LDL, ↓ triglycerides and ↓ IGF-1 expression in liver (highest dose)	
OD-PABA	
Oestrogen activity: In vitro	
↑ transactivation: hERα	Gomez *et al.* (2005) and Schreurs *et al.* (2002)
↑ transactivation: hERα > hERβ,	Schreurs *et al.* (2005)
No agonistic action at hERα, rtERα, hERβ	Kunz *et al.* (2006), Kunz & Fent (2006b), Morohoshi *et al.* (2005) and Schreurs *et al.* (2002)

Table 2 *Continued*

Endpoint	References
OD-PABA	
Antagonism of hERα transactivation	Kunz & Fent (2006b) and Morohoshi *et al.* (2005)
No antagonism at hERα or hERβ	Schreurs *et al.* (2002)
↑ MCF-7 cell proliferation	Schlumpf *et al.* (2001b)
Oestrogen activity: Acute in vivo models	
No uterotrophic effect in immature rats	Schlumpf *et al.* (2001b)
Androgen activity: In vitro	
Antagonism of hAR transactivation	Kunz & Fent (2006b)
No antagonism on hAR transactivation	Ma *et al.* (2003)
No agonistic action hAR transactivation	Kunz & Fent (2006b), Ma *et al.* (2003) and Schreurs *et al.* (2005)
Progesterone activity: In vitro	
No agonism or antagonism on hPR transactivation	Schreurs *et al.* (2005)
PABA	
Oestrogen activity: In vitro	
No binding to hERα	Morohoshi *et al.* (2005)
Antagonism of hERα transactivation	Kunz & Fent (2006b)
No antagonistic action at hERα	Morohoshi *et al.* (2005)
No agonistic actions at hERα, rtERα	Kunz *et al.* (2006), Kunz & Fent (2006b) and Morohoshi *et al.* (2005)
Oestrogen activity: Acute in vivo models	
No data	
Androgen activity: In vitro	
No agonistic or antagonistic activity at hAR, yeast cells	Kunz & Fent (2006b)

oestrogenic in acute in vivo models (Schreurs *et al.*, 2002).

Binding affinity to oestrogen receptor α (ERα) and to oestrogen receptor β (ERβ) has also been examined (Mueller *et al.*, 2003; Schlumpf *et al.*, 2004a; Morohoshi *et al.*, 2005). The studies differed with respect to ERα and ERβ binding preference of individual compounds, but indicate an interaction at the level of ERs. This was also demonstrated by the fact that the proliferative effect of 4-MBC on MCF-7 cells was abolished by the selective ER antagonist ICI182780 (Schlumpf *et al.*, 2001b). Additional effects on oestrogen synthesis, degradation, protein binding, receptor synthesis, etc. cannot be excluded, but have not been investigated as yet.

Interestingly, Kunz and Fent detected antagonistic activity of almost all tested UV-filters in yeast expressing human ERα (hERα): BP-3, 3-BC, 4-MBC, OMC, HMS, OD-PABA and PABA (Kunz & Fent, 2006b). Antioestrogenic activity of 4-MBC, OD-PABA and PABA was supported in a couple of other studies (Mueller *et al.*, 2003; Morohoshi *et al.*, 2005). In contrast, BP-3, found to be the most antioestrogenic UV-filter in the Kunz and Fent study (Kunz & Fent, 2006b) had in another study only weak binding affinity to hERα, compared with 17β-estradiol (Morohoshi *et al.*, 2005). Moreover, Schreurs and colleagues also investigated antagonistic oestrogenic activity of BP-3, 3-BC, 4-MBC, HMS, OMC and OD-PABA, but in contrast, did not report any effects of the tested compounds (Schreurs *et al.*, 2002).

These conflicting results regarding antioestrogenic activity are compatible with data from oestrogen agonist studies indicating that the agonistic activity of many UV-filters is of the partial agonist type (Schlumpf *et al.*, 2001b, 2004a). In addition, those studies differ in their type of assay, being also different in their capability to discriminate between agonistic and antagonistic effects.

The oestrogenic activity of BP-3, 3-BC, 4-MBC and OMC was confirmed by acute in vivo tests using increased uterine weight in immature rats (Schlumpf *et al.*, 2001b, 2004a; Tinwell *et al.*, 2002) or oophorectomized rats (Klammer *et al.*, 2005). Furthermore, elevated vitellogenin in fish, a phenotypic endpoint for the oestrogenic action, has been observed in a number of ecotoxicological studies of 3-BC, 4-MBC and OMC (Holbech *et al.*, 2002; Inui *et al.*, 2003; Kunz *et al.*, 2006). However, increased uterine weight following BP-3 exposure of immature rats conflicted with unchanged uterine weight in an adult oophorectomized rat model, indicating possible higher sensitivity of immature rats to BP-3 (Schlumpf *et al.*, 2001b; Schlecht *et al.*, 2004). In vitro oestrogenic activity of HMS and OD-PABA could not be confirmed in vivo as reported by (Schreurs *et al.*, 2002).

Influence on androgen activity

BP-3, 3-BC, 4-MBC, HMS, OMC and OD-PABA exhibited antiandrogenic activity in vitro, even though data on individual compounds were conflicting (Ma *et al.*, 2003; Schreurs *et al.*, 2005; Kunz & Fent, 2006b). In contrast to other UV-filters, which were mainly androgen antagonists, HMS exhibited both full agonistic and antagonistic androgen activity in vitro by producing full dose-response curve binding to the human androgen receptor (hAR) and inhibiting dihydrotestosterone (DHT) (Kunz & Fent, 2006b). In addition to in vitro antiandrogenic activity, OMC caused a decrease in serum-testosterone among immature offspring in a developmental study in rats (Axelstad *et al.*, 2011). For the remaining compounds in Table 1, antiandrogenic activity of UV-filters has not yet been investigated in vivo.

Influence on progesterone activity

3-BC, 4-MBC, BP-3, OMC and HMS were all tested using a progesterone receptor (PR) CALUX bioassay and all

found to exhibit antagonistic action on the PR in U2-OS cells (Schreurs *et al.*, 2005). The action of those UV-filters could be reversed by the PR agonist ORG2058, indicating a PR mediated action. OD-PABA was also tested, but did not exhibit progesterone activity in vitro (Schreurs *et al.*, 2005).

OMC and 4-MBC were examined for progesterone effect in vivo (Seidlova-Wuttke *et al.*, 2006; Axelstad *et al.*, 2011). Only exposure to OMC in vivo confirmed progesterone activity observed in vitro, resulting in a decrease in the plasma-progesterone concentration in a developmental study in rats (Axelstad *et al.*, 2011) and in altered transcription of PR in uterus and vagina among oophorectomized Sprague–Dawley rats orally exposed for 3 months (Seidlova-Wuttke *et al.*, 2006). In vitro progesterone activity of 4-MBC, was not confirmed in the later study performed on oophorectomized rats (Seidlova-Wuttke *et al.*, 2006). This finding does not rule out the possibility of 4-MBC's interference with progesterone signalling in vivo among immature rats, if their sensitivity is higher compared with oophorectomized rats, just as it was the case for oestrogenic activity of BP-3 (Schlumpf *et al.*, 2001b; Schlecht *et al.*, 2004).

Effects on reproductive organs and development

Studies on reproductive and developmental toxicity have been published for only three of the endocrine active UV-filters, namely 4-MBC, 3-BC and OMC. Delay of male puberty and reduced prostate weight were the most sensitive variables for reproductive toxicity following exposure to 3-BC and 4-MBC in extended one generation developmental studies, where Long Evans rats were orally exposed for 10 weeks before mating, during pregnancy and lactation and then their offspring continued oral exposure until adulthood (Schlumpf *et al.*, 2004b; Durrer *et al.*, 2007). Those effects were seen at a dose of 0.24 mg/kg bw/day for 3-BC and of 7 mg/kg bw/day for 4-MBC. In contrast, the reproductive toxicity two generation study with OMC reported only delayed male and female puberty at the highest dose of 1000 mg/kg bw/day, which was not attributed to the compound, but rather to a natural variation within the 'historical control range', in spite of the statistically significant difference compared with control animals in the study (Schneider *et al.*, 2005). Axelstad *et al.* (2011) did not find any effect of OMC on the time of puberty in a one generation developmental study either.

Several other adverse effects on the reproductive system were observed in extended one generation developmental studies after exposure to BP-3, 3-BC, 4-MBC and OMC comprising alteration in weight and histology of reproductive organs in both sexes (Schlumpf *et al.*, 2004b; Durrer *et al.*, 2007; Hofkamp *et al.*, 2008; Axelstad *et al.*, 2011). Developmental studies with BP-3, 3-BC and 4-MBC (Schlecht *et al.*, 2004; Schlumpf *et al.*, 2004b; Durrer *et al.*, 2005, 2007) and acute and long-term OMC studies in adult oophorectomized rats (Klammer *et al.*, 2005; Seidlova-Wuttke *et al.*, 2006) found alterations in proteins and gene expression of ER, AR, PR, insulin-like growth factor-I (IGF-1), complement protein 3 (C3), nuclear receptor corepressor (N-Cor), steroid receptor coactivator-1 (SRC-1) in uterus and prostate. Those findings indicate the possible mechanism of action behind the reproductive toxicity. Alterations in oestrogen target gene expression following peri- and postnatal exposure with 3-BC and 4-MBC also occurred in brain regions important for rats' sexual behaviour (Ventromedial Hypothalamic nucleus (VMH) and Medial Preoptic area (MPO)) (Maerkel *et al.*, 2005, 2007; Faass *et al.*, 2009). This was supported by observed changes in female sexual behaviour, such as reduction in proceptive behaviour, altered attractive behaviour resulting in a decreased number of mounts, impaired receptive behaviour and episodes of rejection following exposure to 3-BC and 4-MBC in an extended one generation developmental study (Faass *et al.*, 2009).

In addition, a 90-day BP-3 study in adult mice (French, 1992) and a 3-BC extended one generation developmental study in rats (Faass *et al.*, 2009) resulted in changes in the oestrous cycle.

Fertility in males was affected in a 90-day study with BP-3, where sperm density decreased in a dose-related manner following dermal exposure in mice and at the highest dose following oral exposure in mice and rats (French, 1992). In addition, at the same dose level an increased number of abnormal spermatozoa was observed in mice. Perinatal and early postnatal exposure to OMC in rats also resulted in decreased sperm count (Schneider *et al.*, 2005; Axelstad *et al.*, 2011).

Reduction of litter size and survival rate in offspring were seen after exposure of dams during pregnancy to higher doses of 3-BC (above 2.4 mg/kg bw/day) and 4-MBC (above 24 mg/kg bw/day) (Schlumpf *et al.*, 2001a, 2004b). The mechanisms behind this perinatal toxicity have not been clarified, but involvement of the immune system and metabolism of the compounds are suspected because the same doses of 4-MBC caused decrease in thymus weight of offspring and increase in weight of thyroid in dams (Schlumpf *et al.*, 2004b).

Effects on hypothalamic–pituitary–thyroid axis

A wide range of in vitro and in vivo studies support that 4-MBC, BP-3 and OMC may interfere with the hypothalamic–pituitary–thyroid axis (HPT).

An in vitro and a 5-day in vivo study with BP-3 have shown that this compound interacts with thyroid function by an agonistic effect on the thyroid receptor (TR)

in HepG2 cells(Schmutzler *et al.*, 2007b) and by decreasing the expression of the ERR1 gene in the thyroid gland (Schlecht *et al.*, 2004). Whether those findings result in any adverse effect on the thyroid axis have not been examined and further investigations on BP-3 in long-term studies seem indicated.

An adverse effect on the thyroid axis indicated by alterations in the concentrations of thyroid hormones following exposure to 4-MBC and OMC was found in 90 days toxicological studies (Schmutzler *et al.*, 2004, 2007b). An extended one generation developmental study in rats confirmed alterations in thyroid-stimulating hormone (TSH) and total triiodothyronine (T3) following 4-MBC exposure, supplemented with increased thyroid weight in offspring (Maerkel *et al.*, 2007). A developmental study of OMC resulted in decreased total thyroxine (T4) in dams and in juvenile male offspring and in increased weight of thyroid gland in juvenile rats of both sexes (Axelstad *et al.*, 2011). A sex difference was noted: her female offspring were less sensitive to OMC exposure, resulting in unaltered T4 (Axelstad *et al.*, 2011).

The mechanisms behind the adverse effects observed in the thyroid axis following in vivo exposure to 4-MBC and OMC could be partially explained by a decrease in Doi 1 activity, an enzyme promoting both activation and inactivation of thyroid hormones and by decreased iodide uptake in FRTL-5 cells (Klammer *et al.*, 2007; Schmutzler *et al.*, 2007b). In addition, OMC exhibited agonistic action on TR in the HepG2 cell line (Schmutzler *et al.*, 2007b). In contrast, thyroid peroxidise (TPO) activity was not affected neither following OMC exposure nor 4-MBC exposure (Schmutzler *et al.*, 2004; Klammer *et al.*, 2007).

The UV-filter benzophenone-2 (BP-2) is not allowed to be used in cosmetics in EU (European Commission), but is still used in USA to protect cosmetic products against UV-rays (Food and Drug Administration [FDA], 2012). This is of concern as it has been shown to disturb thyroid function in an acute toxicity study on oophorectomized Sprague Dawley rats, altering TSH, T4 and Doi-1 activity and to decrease human receptor TPO (hrTPO) activity in vitro (Schmutzler *et al.*, 2007a).

Several UV-filters, including 3-BC, HMS, OD-PABA or PABA seem not to have been examined for their possible effects on the thyroid axis.

General toxicity

In mammalian long-term exposure models, general toxicity evaluated by alterations of food consumption and in body and liver weights was found in the higher dose range after exposure to BP-3 (>2.4 mg/kg bw/day) and OMC (>500mg/kg bw/day) (French, 1992; Schlumpf *et al.*, 2004b; Schneider *et al.*, 2005; Axelstad *et al.*, 2011). Liver and kidney weights were affected after both dermal and oral exposure to BP-3 (French, 1992). In contrast, histological alterations in liver and kidney were observed only after oral exposure to BP-3 in the latter study. Four-MBC reduced body weight only transiently in postnatal 4-MBC- exposed F1 offspring. Body weights were again normal at puberty and in adulthood also in the higher dose groups, and no signs of general toxicity were observed in the parent animals (F0) (Durrer *et al.*, 2007; Maerkel *et al.*, 2007). Effects on reproductive organs and development were also present at doses devoid of general toxicity. Indications of general toxicity were further observed in experiments on fish exposed to 3-BC and 4-MBC, where body weight decreased in a dose-dependent manner (Kunz *et al.*, 2006). A few deaths were observed by Schneider and colleges in a two generation study in rats, but were not considered to be related to OMC exposure (Schneider *et al.*, 2005).

Human exposure to sunscreens

Table 3 summarizes prevailing data on human exposure to chemical UV-filters used in cosmetics. Experimental studies showed that BP-3, 4-MBC and OMC rapidly permeated intact skin (Gustavsson *et al.*, 2002; Janjua *et al.*, 2004, 2008; Gonzalez *et al.*, 2006) and could be detected in plasma after 1–2 h following application (Fig. 2) (Janjua *et al.*, 2008). Interestingly, the concentrations of these compounds in the same experimental study in male urine and plasma were higher than in female samples (Janjua *et al.*, 2004), indicating a gender difference in the metabolism, distribution and possibly also in the accumulation of UV-filters in adipose tissue.

Furthermore, Table 3 shows a substantial exposure of the general population to UV-filters. BP-3, the most common UV-filter in the USA, was found in more than 96% of 2517 urine samples collected throughout 1-year (2003–2004) from the general US population in an NHANES study (Calafat *et al.*, 2008). BP-3 was also detected in all urine samples collected from 129 Danish children and adolescents in the month of November, even though days are short and sun protection is not needed at that time of year (H. Frederiksen, O. Nielsen, L. Aksglaede, K. Sorensen, T. H. Lassen, N. E. Skakkebaek, K. Main, A. Juul & A. Andersson, unpublished data, 2012).

Breastfed babies are exposed to UV-filters through breast milk (Schlumpf *et al.*, 2010). One or more UV-filters were present in 85% of Swiss human milk samples (Schlumpf *et al.*, 2010). Bisphenol A with a similar chemical structure to BP-3, were shown to pass the blood-placenta barrier (Schonfelder *et al.*, 2002; Lee *et al.*, 2008). Thus in theory, chemicals like BP-3 may also pass the blood-placenta barrier. Studies investigating amniotic fluid are required to investigate whether perinatal exposure to UV-filters, which

Table 3 Human exposure to UV-filters

	Study design	Number of test subjects	Sample type	UV-filter	Reported use(%)	Positive samples(%)	Concentrations		Ref.
Observational studies	*USA*: NHANES study 2003–2004 based on US general population > 6 years of age	2517 people	Urine	BP-3	–	96.8	22.9 (18.1–28.9) μg/L[c]		Calafat *et al.* (2008)
	USA: The Children's Environmental Health study 1998–2002: Multiethnic prospective cohort of pregnant women-infant pairs in New York during 3. trimester	404 pregnant women	Urine	BP-3	–	97.8	7.5 μg/L[a]		Wolff *et al.* (2008)
	France: Eden Mother-Child cohort recruited before gestational week 28 in 2003–2006	191 pregnant women	Urine	BP-3	–	80.5	1.3 μg/L[a]		Philippat *et al.* (2012)
	Schweiz: Cohorts 2004–2006 Mothers who gave birth to a single child at the Univer sity Women's Hospital Basel	54 women	Human breast milk	BP-3	13.21	12.96	52.23 ± 50.69 ng/g lipid		Schlumpf *et al.* (2010)
				4-MBC	26.42	20.37	22.12 ± 12.80 ng/g lipid		
				OMC	66.04	77.78	27.50 ± 22.15 ng/g lipid		
				HMS	15.09	5.56	29.37 ± 27.64 ng/g lipid		
				OCT	43.40	66.67	30.18 ± 24.51 ng/g lipid		
				OD-PABA	1.89	1.85	49.00 ng/g lipid[c]		
				3-BC	0	0	–		
Experimental studies	*Denmark*: Single blinded experimental study 1 week with UV-filters free lotion + 1 week with Daily whole-body application of sunscreen 2 mg/cm^2 with BP-3, 4-MBC and OMC Concentration 10% of each	32: 15 males + 17 postmenopausal females	Plasma		100	100	Female (ng/ml)	Male (ng/ml)	Janjua *et al.* (2004)
				BP-3			200[b]	300[b]	
				4-MBC			20[b]	20[b]	
				OMC			10[b]	20[b]	
			Urine	BP-3			60[b]	140[b]	
				4-MBC			5[b]	7[b]	
				OMC			5[b]	8[b]	
			Plasma	BP-3			187[b]	238[b]	Janjua *et al.* (2008)
				4-MBC			16[b]	18[b]	
				OMC			7[b]	16[b]	
			Urine	BP-3			44[b]	81[b]	
				4-MBC			4[b]	4[b]	
				OMC			6[b]	4[b]	
	Sweden: Experimental study 1x whole-body application of sunscreen with BP-3 2mg/cm^2 Concentration 4%	11: 7 males + 4 females	Urine	BP-3	100	100	9.8 mg = 0.5% of the applied amount		Gustavsson *et al.* (2002)
	Sweden: Experimental study: hole-body application of 2 mg/cm^2 sunscreen SPF14 with 4% BP-3 twice a day for 5 days One half was daily irradiated with UVA+UVB according to Fitzpatrick skin type; 16 women and 9 men	25: 16 women and 9 men	Urine:	BP-3	100	100	3.7% of applied amount		Gonzalez *et al.* (2006)

[a]Median concentration.
[b]*Maximum median concentration.
[c]Geometric mean concentration.

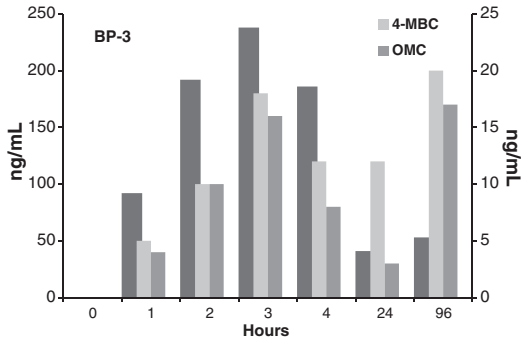

Figure 2 Plasma concentration of UV-filters BP-3, 4-MBC and OMC among males after one dermal application daily (Janjua *et al.*, 2008).

are found in the urine of pregnant women, occurs (Wolff *et al.*, 2008). High concentrations of BP-3 in mothers' urine were associated with decreased birth weight in girls and increased birth weight and head circumference in boys (Wolff *et al.*, 2008; Philippat *et al.*, 2012). Human studies with genital malformations in infants as an endocrine specific endpoint are required to clarify a possible endocrine disrupting effect of UV-filters on the human foetus. However, developmental animal studies where rats were exposed to UV-filters, did not report any reduction of the anogenital distance (ADG) or increased rate of genital malformations.

In spite of the wide human exposure to UV-filters only few studies have examined the effects of UV-filters on humans (Janjua *et al.*, 2004, 2007; Jannesson *et al.*, 2004; Wolff *et al.*, 2008; Philippat *et al.*, 2012). A double blinded clinical trial measuring the gingival index, that relate to the severity and location of periodontal disease, showed that dentifrice containing BP-3 reduced periodontal disease by 25% (Jannesson *et al.*, 2004), which supported the in vitro data suggesting BP-3 to be an inhibitor of PG synthesis (Jannesson *et al.*, 2004; Kristensen *et al.*, 2011).

Janjua and colleagues examined the effects on reproductive (Janjua *et al.*, 2004) and thyroid (Janjua *et al.*, 2007) hormones following dermal application of a mixture of BP-3, 4-MBC and OMC. A significant increase in inhibin B and a decrease in free triiodothyronine (FT3), free thyroxine (FT4), T3, T4, thyroxine-binding globulin (TBG) and testosterone were seen, but were not considered to be related to the application of a sunscreen mixture, but rather to biological variation (Janjua *et al.*, 2007). However, the duration of those studies was too short to be conclusive.

Discussion

As summarized in this review, a large number of in vivo animal studies and in vitro studies have shown that there are numerous potential adverse effects of UV-filters present in sunscreens and cosmetics. The effects include developmental and reproductive effects, apparently caused by endocrine disrupting actions of these chemicals. Other studies could not find such adverse effects. However, because of the wide human exposure in combination with the clear endocrine disruptive effects observed in a large number of well designed studies, the UV-filters BP-3, 4-MBC and OMC can be considered as substances of high concern in relation to human risk.

Importantly, most of the studied adverse effects of UV-filters have been evaluated after oral exposure. However, the primary exposure of humans to UV-filters via cosmetics occurs through dermal application. Therefore, the UV-filters enter the systemic circulation directly without first being metabolized by passage through the liver, thereby leading to a greater risk of the compounds reaching all tissues of the body unaltered, as was observed in rats following dermal exposure to 3-BC (Søeborg *et al.*, 2006). In addition, a three-fold greater oestrogenic effect of 4-MBC in rats was observed after topical application compared with oral exposure indicating higher bioavailability of the compound (Schlumpf *et al.*, 2001b).

Another challenge in studies of sunscreens in cosmetics is that the products often contain several UV-filters in combination. The total effect of these mixtures are poorly examined although a few existing studies have shown that mixtures of chemicals, including UV-filters, might act additively and exhibit toxic activity, even at the No Observed Adverse Effect Level (NOAEL) of the individual compounds (Heneweer *et al.*, 2005; Kunz & Fent, 2006a; Kortenkamp *et al.*, 2007).

Humans are not only exposed to UV-filters when the agents are used for sun protection of the skin. Exposure apparently occurs from various sources. Almost all samples in an NHANES study (Calafat *et al.*, 2008) and all samples in a Danish children cohort (Frederiksen H. *et al.*, in preparation) contained BP-3, indicating year round exposure independent of sunscreen use. The presence of UV-filters in milk of Swiss mothers was correlated with use of sunscreens in 55% of the cases; in 60% of the cases, the presence of these compounds in milk was related to the use of other cosmetic products containing UV-filters (Schlumpf *et al.*, 2010). The likely sources may be hair spray, lipsticks, shampoo, make-up, perfumes, skin care products as well as non-cosmetic products, such as carpets, furniture, clothing and washing powder (Schlecht *et al.*, 2004; Morohoshi *et al.*, 2005; Kunz & Fent, 2006b; Schlumpf *et al.*, 2010). Here, the UV-filters are used to protect the products from effects of UV-radiation. Considering these findings, it cannot be ruled out that a considerable part of the total human exposure to UV-filters might occur via products other than sunscreens.

It is of particular concern that human babies are exposed to UV-filters through breast milk (Schlumpf *et al.*, 2010). The highest concentration of 4-MBC found in human milk was 48.37 ng/g lipid (Schlumpf *et al.*, 2010), which was only 4.3 times lower than the concentration of 4-MBC in rat milk (208.6 ng/g lipid) (Schlumpf *et al.*, 2008) following oral exposure to 4-MBC at the Lowest Observed Adverse Effect Level (LOAEL) (7 mg/kg/day), with delay of male puberty and prostate weight as endpoints (Durrer *et al.*, 2007).

In conclusion, it is of concern that (1) a large number of in vitro and in vivo animal studies have shown endocrine disrupting effects of UV-filters present in sunscreens, although other studies failed to find such effects and (2) application of cosmetics with UV-filters to the skin can result in absorption of UV-filters into the human systemic circulation and subsequently might result in exposure of all tissues in the body. Considering these facts together with the wide and increasing use of sunscreens and the increasing incidence of malignant melanoma, for which UV-filters are assumed to protect, it seems pertinent to investigate whether sunscreen use in humans on balance is beneficial for human health.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Purchase of sunscreen products by volume per head over time in the US, UK, AUS and DK.

Table S1. In vitro and in vivo effects of UV-filters in animals (Presence of effect: +; Absence of effect: −; Increased: ↑; Decreased: ↓).

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