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Endocrine activity and developmental toxicity of cosmetic UV filters—an update

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Abstract

UV filters represent a new class of endocrine active chemicals. In vitro, 8/9 chemicals showed estrogenic (MCF-7 cells), and 2/9 antiandrogenic activity (MDA-kb2 cells). Six/nine filters (benzophenone (Bp)-1, Bp-2, Bp-3, 3-benzylidene camphor (3-BC), 4-methylbenzylidene camphor (4-MBC), octyl-methoxycinnamate (OMC)) increased uterine weight in immature rats. 3-Benzylidene camphor and 4-MBC displaced 16α^{125}I-estradiol from human estrogen receptor (ER)β , not ERα. Developmental toxicity of 4-MBC (0.7–47 mg/kg body weight/day) and 3-BC (0.24–7 mg/kg), administered in chow was investigated in Long Evans (LE) rats. Weight gain of pregnant rats was reduced only by 3-BC, early postnatal survival rate and thymus weight by both compounds at higher doses. 4-Methylbenzylidene camphor and 3-BC delayed male puberty, and dose-dependently affected reproductive organ weights of adult male and female F1 offspring, with partly different effect patterns. Thyroid weight was increased by higher 4-MBC doses. Tissue-specific changes in mRNA levels of estrogen-regulated genes in prostate, uterus and brain regions, determined by real-time PCR, and in their response to acute estradiol challenge in adult gonadectomized offspring were observed. Lowest effective doses were 0.24 mg/kg/day for 3-BC and 7 mg/kg/day for 4-MBC. Fat tissue levels at 7 mg/kg 4-MBC (GC–MS) approached the range of UV filters in fish (Nagtegaal et al., 1997; Balmer et al., 2004).

Keywords: UV filters; 4-Methylbenzylidene camphor (4-MBC); 3-Benzylidene camphor (3-BC); Estrogenic; Development; Toxicity; Gene expression

1. Introduction

UV filters are lipophilic high production volume substances with an increasing diverse spectrum of use,

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as sunscreens or product protection in cosmetics, as additives in plastics, folia, carpets, furnish, clothing and washing powder. UV Filters have to be declared in cosmetics but remain essentially unidentified in technical products. Toxicology and kinetics, behavior and modes of interaction with the ecosphere are unknown or unpublished. Possible exposure scenarios are many fold as humans and animals can be exposed through the food chain and humans through skin. Starting to analyze toxicology of cosmetic UV filters on endocrine activity and developmental toxicity seems like an enormous, but necessary task vis-a-vis the approximate 10,000 chemicals used by cosmetic industry. In a first step, we investigated for possible endocrine activities in vitro and in vivo, and then went on to study effects in a developmental toxicity test for evaluating possible long-term risks.

2. Materials and methods

2.1. Identification of endocrine activity in vivo and in vitro and estrogen receptor binding

Estrogenic activity in vitro was studied in MCF-7 cells kindly provided by A. Soto (Tufts University, Boston, USA) according to previously published methods (Schlumpf et al., 2001a).

Androgenic and antiandrogenic activity was analysed in MDA-kb2 cells kindly provided by K. Bobseine and L.E. Gray (Endocrinology Branch US EPA) (Ma et al., 2003).

Subtype-specific estrogen receptor (ER) ligand binding (ER-LBA) was performed according to Jarry et al. (2003), using recombinant human ERα and ERβ obtained from Panvera (Madison, USA) (Schlumpf et al., 2004). Competition experiments were performed with 16α^{125}I-estradiol (2200 Ci/mmol, New England Nuclear, Dreieich, Germany) as radioligand.

Estrogenic activity in vivo was determined using the uterotrophic assay in immature rats. In the first study series on Long Evans (LE) rats from Mρllegard (Denmark), chemicals were admixed to the chow (Schlumpf et al., 2001a). More recently, LE rats from Centre d'Elevage R. Janvier (France) were given the chemicals by oral gavage on 3 days, postnatal day (PN) 21, PN22 and PN23 (PN1: day of birth), and sacrificed 24 h later, according to the OECD protocol. Chemicals were dissolved in olive oil (0.04 ml/10 g body weight; vehicle control: olive oil). In the dose range studied (2–1200 mg/kg bw), the UV filters showed no overt toxicity. Doses were not further increased if the animals did not gain weight or gained weight considerably less than controls

2.2. Developmental toxicity: 4-methylbenzylidene camphor (4-MBC) and 3-benzylidene camphor (3-BC)

Long Evans rats were bred in our animal facilities under standard conditions (lights 0200–16.00 h, 22 °C ± 1 °C) with food and water ad libitum. Males and females of the parent generation (F0) were exposed to 4-methylbenzylidene camphor (4-MBC) or 3-benzylidene camphor (3-BC) for at least 10 weeks before mating. Exposure of dams continued through pregnancy and lactation and for the offspring (F1) until adulthood. The compounds were added to chow 3430 (Provimi Kliba AG, Kaisraugst, Switzerland) at the following concentrations: 4-MBC 0.01, 0.1, 0.33 and 0.66 g/kg chow yielding an average daily intake of 0.7, 7, 24 and 47 mg/kg body weight (bw). 3-BC 0.0033, 0.01, 0.033 and 0.1 g/kg chow yielding an average daily intake of 0.24, 0.7, 2.4 and 7 mg/kg bw. The 4-MBC study was started with a dose of 70 mg/kg/day (1.0 g/kg chow), corresponding to 60% of the minimally effective dose in the uterotrophic assay. This dose was well tolerated in adult F0 rats but markedly reduced postnatal survival, and therefore was discontinued. 3-BC doses were chosen according to the potency difference between 4-MBC and 3-BC in the uterotrophic assay, with one overlapping dose (7 mg/kg/day).

Several developmental toxicity parameters were monitored, such as weight gain of male and female F0 rats and of pregnant dams, birth weight (birth at gestational day 23 = postnatal day PN 1), litter size (counted and adjusted to 8–10 pups at PN 2), survival rate at postnatal days (PN) 2–14, onset of puberty (females: vaginal opening, males: preputial separation), weight of reproductive organs (ovary, uterus, testis, epididymis, seminal vesicles, ventral and dorsal prostate) and non-reproductive organs (i.e. thymus, thyroid, liver). Offspring were sacrificed by decapitation (females in diestrus) under light ether anesthesia. Organs were stored in liquid nitrogen. Brains were frozen on dry ice and stored at −80 °C until dissection in a matrix (Bilany RBM 3000C). Two

sexually dimorphic brain regions, medial preoptic area (MPO) and ventromedial hypothalamic nucleus (VMH), were punched out of 1 mm thick coronal brain sections at defined antero-posterior levels.

2.3. Real-time PCR

Following tissue disruption with RLT buffer, total RNA of peripheral organs and regions of the brain were isolated using RNeasy mini kit (QUIAGEN, 4052 Basel, Switzerland) (Maerkel et al., in press). DNA contamination was checked on an ethidium bromide-stained 2.5% agarose gel. TaqMan reverse transcription reagents kit, (Applied Biosystems, 8343 Rotkreuz, Switzerland) was used to obtain cDNA from total RNA. mRNA levels of ERα and ERβ, progesterone receptor (PR), androgen receptor (AR), insulin like growth factor-I (IGF-I), and, in brain, pre-proenkephalin (PPE) were quantified by real-time PCR using TaqMan universal PCR master mix (Applied Biosystems) and the ABI PRISM 7700 sequence detection system (Applied Biosystems). mRNA sequences were derived from NCBI (National Center for Biotechnology Information) gene bank. TaqMan probes and primers were designed with Primer Express Software, Version 2.0 (Applied Biosystems) and ordered from Microsynth (9436 Balgach, Switzerland). mRNA levels were calculated using the relative standard curve method, with cyclophilin A as a reference gene.

2.4. GC–MS analysis of 4-MBC

Approximately 0.1 g of adipose tissue was homogenized in a mortar together with ca. 0.5 g anhydrous sodium sulphate. The mixture was packed in a Pasteur pipette plugged with glass wool. The lipids were eluted with 10 ml *n*-hexane. After adding the internal standard (3-BC), an aliquot of 10% of the extract was applied to a Pasteur pipette packed with glass wool, 0.5 g silica gel activated at 130 °C and 0.25 g anhydrous sodium sulphate. A first fraction to be discarded was eluted with 10 ml of *n*-hexane/dichloromethane (98:2, v/v). The second fraction eluted with 10 ml of *n*-hexane/dichloromethane (1:1, v/v) contained the analytes (4-MBC and the internal standard (3-BC)). After addition of 100 μl toluene and volume reduction to 100 μl in a rotary evaporator, the sample was ready for GC–MS analysis.

2.5. Statistical analysis

Treatment groups were compared by analysis of variance (ANOVA) followed by pairwise comparisons with Bonferroni correction. Non-normally distributed values (e.g., puberty) were analyzed by non-parametric methods (Kruskal–Wallis and Mann–Whitney *U*-test).

3. Results and discussion

3.1. Identification of endocrine activity in vitro

So far, 10 UV filters have been tested in vitro (Table 1), benzophenone-1 (Bp-1), benzophenone-2 (Bp-2), benzophenone-3 (Bp-3). benzophenone-4 (Bp-4), 3-benzylidene camphor (3-BC), 4-MBC, homosalate (HMS), octyl-dimethyl-PABA (OD-PABA), OMC, and butyl-methoxydibenzoyl-methane (BMDM).

3.2. Estrogenic activity and estrogen receptor binding

All of these UV filters except Bp-4, were screened for estrogenic activity on MCF-7 cells (E SCREEN), with cell proliferation and pS2 protein as endpoints. Eight of nine chemicals. showed estrogenic activity, one, BMDM, was ineffective (Table 1. Schlumpf et al., 2001a and in preparation). In competition experiments with 16α^{125}I-estradiol on recombinant human ERβ, two UV filters, 4-MBC and 3-BC, were identified as ERβ ligands, whereas no displacement of the radioligand was seen on human ERα at concentrations up to 1 mM (Schlumpf et al., 2004). Additional UV filters are presently under investigation.

EC50 values of estrogenic UV filters are within the low micromolar range and similar to values known for other environmental chemicals identified as xenoestrogens. (for reference, Schlumpf et al., 2001a). The proliferative activity of 4-MBC in MCF-7 cells was confirmed by Tinwell et al. (2002), and the compound was shown to activate transcription in human cell lines expressing ERα or ERβ (Schreurs et al., 2002; Mueller et al., 2003). The debate on the relative potency of different chemicals has not been closed. Results appear to depend considerably on the in vitro system used. In a yeast cell system, 4-MBC was virtually ineffective (Tinwell et al., 2002).

Table 1
Estrogenic and antiandrogenic activities and estrogen receptor α (ERα) and ERβ binding of UV filters

Chemical	Estrogenic activity EC50 (MCF-7 cells)	ERα and ERβ receptor Binding IC50	Antiandrogenic activity IC50 (MDA-kb2 cells)
Benzophenone-1 (Bp-1)	2.08 μM		Not tested
Benzophenone-2 (Bp-2)	0.68 μM		Not tested
Benzophenone-3 (Bp-3)	3.73 μM		4.98 μM (in 0.1 nM DHT)
Benzophenone-4 (Bp-4)	Not tested		No effect
3-Benzylidene camphor (3-BC)	0.68 μM	ERβ 11.8 μM ERα No displacement[a]	No effect
4-Methylbenzylidene camphor (4-MBC)	3.02 μM	ERβ 35.3 μM ERα No displacement[a]	No effect
Homosalate (HMS)	1.56 μM		5.57 μM (in 0.1 nM DHT)
Octyl-dimethyl PABA (OD-PABA)	2.63 μM		No effect
Octyl-methoxycinnamate (OMC)	2.37 μM		No effect
Butyl-methoxydibenzoyl-methane (BMDM)	No effect		No effect

References: Estrogenic activity: Schlumpf et al., 2001a, Schlumpf et al., 2004 (3-BC) and in preparation (Bp-1, Bp-2, Bp-4). Estrogen receptor binding: Schlumpf et al., 2004. Antiandrogenic activity: Ma et al., 2003.
[a] ERα: No displacement up to 1 mM 3-BC and 4-MBC.

3.3. Antiandrogenic activity in MDA-kb2 cells

Eight UV filters were analysed for inducing luciferase activity in MDA-kb2 breast cancer cells stably transfected with the luciferase reporter gene (Table 1): Two UV filters out of 8 tested antagonized dihydrotestosterone (DHT)-induced luciferase activation. None of the eight filters exhibited androgenic activity (Ma et al., 2003). IC50 values place the two antiandrogenic UV filters at about the level of activity of *p,p′*-DDE, between linuron and the more active vinclozolin.

3.4. Identification of estrogenic activity in vivo (uterotrophic assay)

Chemicals exhibiting estrogenic activity in vitro were examined in the in vivo uterotrophic test on immature rats (Table 2). Six UV filters out of nine tested increased uterine weight in immature rats, with ethinylestradiol (EE) as positive control (Schlumpf et al., 2001a, Schlumpf et al., 2004 and in preparation): Bp-1, Bp-2, Bp-3, 3-BC, 4-MBC, and OMC, while BMDM, HMS and OD-PABA were inactive. BMDM, Bp-3, HMS, 4-MBC, OD-PABA and OMC were tested by application of the compound in the chow, 3-BC, Bp-1 and Bp-2 were administered by oral gavage. For comparison, 4-MBC was repeated by gavage, with analogous results (Schlumpf et al., 2004). The uterotrophic activity of 4-MBC has been confirmed by Tinwell et al. (2002).

The two camphor derivatives, 3-BC and 4-MBC (and Bp-2), display higher estrogenic activities in vivo than the other chemicals tested. 3-BC is the most potent compound tested so far, being between 6 and 7 times more active than 4-MBC in vitro and in vivo. It displays estrogenic activity not only in mammals, but also in an in vivo fish assay (Holbech et al., 2002). The

Table 2
In vivo estrogenic effect (uterotrophic assay)

Chemical	ED50 (mg/kg body weight/day)
3-Benzylidene camphor (3-BC)	45
4-Methylbenzylidene camphor (4-MBC)	309
Octyl-methoxycinnamate (OMC)	934
Benzophenone-3 (BP-3)	>1000
Benzophenone-1 (Bp-1)	Positive; uterotrophic effect ongoing analysis
Benzophenone-2 (Bp-2)	Positive uterotrophic effect ongoing analysis
Homosalate (HMS)	Inactive
Octyl-dimethyl PABA (OD-PABA)	Inactive
Butyl-methoxydibenzoyl-methane (BMDM)	Inactive

References: Schlumpf et al., 2001a; Schlumpf et al., 2004 (3-BC), and in preparation.

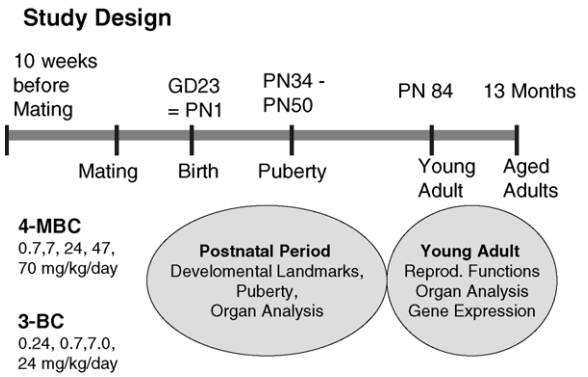


Fig. 1. Study design of developmental toxicity studies with 4-methylbenzylidene camphor (4-MBC) and 3-benzylidene camphor (3-BC).

uterotrophic activity of 3-BC and 4-MBC may seem surprising in view of the virtual absence of binding of the two compounds to ERα. According to indirect evidence from immature ERβ knockout mice, ERβ activation might rather have antiproliferative effects in uterus (Weihua et al., 2000). 3-BC given by the oral route is almost as active in the uterotrophic assay as subcutaneous genistein (Yamasaki et al., 2002), a preferential ERβ ligand with significant transactivational activity at ERα (Kuiper et al., 1997). Whether the uterotrophic activity of 3-BC and 4-MBC is really due to their affinity for ERβ, remains to be clarified; alternatively, the two compounds (or possible metabolites) might exert some transactivational activity at ERα that cannot be assessed from binding studies.

3.5. *Developmental toxicity of 4-MBC and 3-BC*

4-Methylbenzylidene camphor or 3-BC were administered in chow to Long Evans rats from at least 10 weeks before mating of the parent generation, during pregnancy and lactation, and to the F1 generation until adulthood (Fig. 1). Weight gain of pregnant rats was dose-dependently reduced by 3-BC, but not 4-MBC. Litter size and survival rate during the first two weeks of life were decreased by both, 4-MBC and 3-BC, in the higher dose range (Table 3a and Table 3b) (Schlumpf et al., 2001b, and in preparation). 4-MBC affected survival of females to a higher degree than that of males, whereas no comparable sex difference was evident with 3-BC. The cause of the perinatal toxicity is not known. Animals of the F0 generation, exposed only in adulthood, did not exhibit overt signs of toxicity even with the highest doses of 4-MBC after exposure until old adult age. One organ that appears to be affected by chronic 4-MBC in F0 as well as F1 animals, is the thyroid which exhibits an increase in weight at higher 4-MBC doses in both sexes (Table 3a), however, the effect was more pronounced in adult F1 than F0 rats. It cannot be excluded that perinatal disturbances in thyroid activity might influence survival. The neonatal immune system is also influenced by the camphor derivatives. PN 14 thymus weight was reduced by 24 and 47 mg/kg 4-MBC and 7 mg/kg 3-BC.

The onset of puberty was found to be one of the most sensitive parameters. The lowest dose to delay male puberty (preputial separation) was 7 mg/kg for 4-MBC and as low as 0.24 mg/kg for 3-BC (Table 3a and 3b). In contrast, the onset of female puberty (vaginal opening) was not significantly affected (Lichtensteiger et al., 2002, and in preparation). It is interesting to note that treatment with the preferential ERβ ligand genistein during pregnancy and lactation can also result in delayed preputial separation (Wisniewski et al., 2003). Yet, data on genistein are conflicting and appear to depend, i.a., on treatment window and dose (Levy et al., 1995; Nagao et al., 2001).

Body weight of adult offspring was unchanged after 4-MBC but reduced by 3-BC, particularly in females (Table 3a and 3b). Significant 4-MBC- and 3-BC-induced changes in weights of reproductive organs were observed in adult, developmentally exposed F1 offspring. Ventral prostate weight represented a sensitive parameter. It decreased as a result of treatment by both compounds in the lower dose range (Table 3a and 3b). In the case of 3-BC, the change in weight was limited to the lowest dose, 0.24 mg/kg, but alterations in gene expression were observed also in the absence of weight changes (Fig. 2). Differences in the effect patterns of the two camphor derivatives were noted in other organs (Table 3a and 3b). 4-MBC affected testes weight with a decrease of absolute and relative weights at postnatal day 14 (Schlumpf et al., 2001b) and an increase (absolute weight from 7 mg/kg, relative weight at 47 mg/kg) in adult (12 week-old) offspring, whereas no changes in adult testes weight were seen after 3-BC. Epididymis and seminal vesicle weight was affected by 3-BC rather than 4-MBC. Uterine weight was elevated after exposure to 4-MBC but decreased by the high-

Table 3a
4-Methylbenzylidene camphor (4-MBC): synopsis of significant changes in selected endpoints of F1 rat offspring at 12 weeks of age after pre- and postnatal exposure in chow

Endpoint	4-Methylbenzylidene camphor mg/kg/day p.o.			
	0.7	7	24	47
Perinatal period[a]				
Litter size	Ø.	Ø	Decreased	Decreased
Survival rate	Ø	Ø	Decreased	Decreased
Thymus weight (relative), males and females PN1[b]	n.t.	Ø	Decreased	Decreased
Testis weight (relative), PN14[b]	n.t.	Decreased	Decreased	Decreased
Puberty[a]				
Males (preputial separation)	n.t.	Delayed	Delayed	Delayed
Females (vaginal opening)	Ø	Ø	Ø	Ø
Adult F1 organ weights[a,c] (relative weights)				
Male body Weight	Ø	Ø	Ø	Ø
Testis	n.t.	Ø	Ø	Increased
Ventral prostate	Ø	Decreased	Decreased	Decreased
Thyroid males	n.t.	Ø	Increased	Increased
Thymus males	n.t.	Ø	Decreased	Decreased
Female body weight	Ø	Ø	Ø	Ø
Uterus	Ø.	Increased	Increased	Ø
Ovary	Ø	Ø	Increased	Increased
Thyroid females	n.t.	Ø	Increased	Increased
Thymus females	n.t.	Ø	Ø	Decreased
Adult mRNA levels of estrogen-regulated genes[c,d] Progesterone receptor and IGF-I				
Progesterone receptor mRNA				
Uterus		Ø	Decreased	Decreased
Ventromedial hypothalamus, female		Decreased	Decreased	Decreased
Ventromedial hypothalamus, male		Ø	Ø	Ø
IGF-I mRNA				
Uterus		Increased	Ø	Decreased
Dorsal prostate		Ø	Decreased	Decreased
Sensitivity of estrogen-regulated genes to acute estradiol challenge[d,e] progesterone receptor				
Progesterone receptor mRNA		Response to estradiol		
Uterus		Decreased	Decreased	n.t.
Ventromedial hypothalamus, male		Increased	Increased	n.t.
Ventromedial hypothalamus, female		Increased	Ø	n.t.

Ø: no statistically significant effect. n.t.: not tested.
[a] Schlumpf et al., 2001b, and in preparation, Lichtensteiger et al., 2002.
[b] PN 1 : day of birth.
[c] 12 Week-old F1 rat offspring under steady state conditions, females in diestrus.
[d] Durrer et al., in preparation, Maerkel et al., in press, Lichtensteiger et al., 2002.
[e] 6 h after 10 or 50 μg/kg estradiol s.c. in adult 12 week-old offspring gonadectomized at 10 weeks.

est dose of 3-BC, while ovary weight was increased only by 4-MBC exposure. Why effect patterns differ between 4-MBC and 3-BC in spite of the similar actions of the two chemicals in acute assays and similar ER binding characteristics, is not known. Differences in dose-response characteristics or in the formation of metabolites might play a role.

3.6. Estrogen target gene expression in brain and reproductive organs of adult offspring

mRNA levels of six estrogen-regulated genes were determined in brain and reproductive organs of adult male and female offspring by real-time PCR, with cyclophilin as reference gene (Table 3a). Following de-

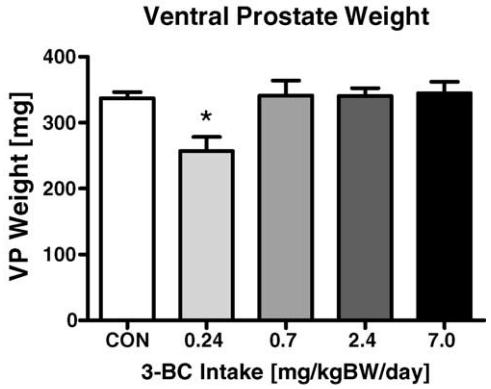
Table 3b
3-Benzylidene camphor (3-BC): synopsis of significant changes in selected endpoints of F1 rat offspring at 12 weeks of age after pre- and postnatal exposure in chow

Endpoint	3-Benzylidene camphor mg/kg/day p.o.			
	0.24	0.7	2.4	7.0
Perinatal Period[a]				
Litter size	Ø	Ø	Ø	Decreased
Survival rate	Ø	Ø	Decreased	Decreased
Puberty[a]				
Males (preputial separation)	Delayed	Ø	Delayed	Delayed
Females (vaginal opening	Ø	Ø	Ø	Ø
Adult organ weights (relative weights)[a,b]				
Male Body Weight	Ø	Ø	Ø	Decreased
Testis	n.t.	n.t.	Ø	Ø
Epididymis	n.t.	n.t.	Decreased	Ø
Seminal Vesicle	n.t.	n.t.	Decreased	Ø
Ventral Prostate	Decreased	Ø	Ø	Ø
Thymus, males	n.t.	n.t.	Ø	Ø
Female Body Weight	Increased	Ø	Decreased	Decreased
Uterus	Ø	Ø	Ø	Decreased
Ovary	n.t.	n.t.	Ø	Ø
Thymus females	n.t.	n.t.	Ø	Increased

Ø: no statistically significant effect. n.t.: not tested.
[a] Schlumpf et al., in preparation.
[b] 12 Week-old F1 rat offspring under steady state conditions, females in diestrus.

velopmental exposure to 4-MBC, significant changes were observed under steady state conditions (females in diestrus) in uterus of adult (12 week-old) offspring (mRNAs encoding for IGF-I, ERα, PR, AR), and in ventral and dorsal lobes of prostate (IGF-I, ERα, ERβ. AR). In order to assess for possible changes in sensitivity to estrogen, acute estrogen challenge experiments were conducted. Adult 4-MBC-exposed offspring were gonadectomized on PN70, subcutaneously injected with either 10 or 50 μg/kg estradiol on PN84, and analyzed 6 h after the injection. Data indicate changes in estrogen sensitivity including lower sensitivity of PR in uterus (Table 3a. S. Durrer et al., in preparation). Developmental exposure to 3-BC also resulted in changes in estrogen target gene expression. Changes in AR mRNA levels of ventral prostate are shown in Fig. 2.

At central nervous system level, we studied gene expression in ventromedial hypothalamic nucleus (VMN) and medial preoptic area, which belong to the network of sexually dimorphic brain regions involved in the integration of environmental cues, sexual behavior and control of gonadal function (Segovia et al., 1993). mRNAs encoding for PR and pre-proenkephalin, the precursor of the neuropeptide enkephalin, are induced in these regions by natural estrogens, with PPE mRNA levels in VMN positively correlated to sexual behavior (lordosis response) of female rats (Brown et al., 1987; Lauber et al., 1990, 1991). Female sexual behavior depends on the induction of the two mRNA species, as demonstrated by blockade of the lordosis response by injection of antisense oligonucleotides for either PR mRNA or PPE mRNA into the ventomedial hypothalamus during the phase of induction by estrogen (Ogawa et al., 1994; Nicot et al., 1997). 4-MBC-exposed adult F1 offspring exhibited sex- and region-specific changes in mRNAs encoding for PR, PPE as well as ERα. The sexual dimorphism of PR mRNA in VMN, with higher levels in females, was abolished by developmental 4-MBC exposure (7 mg/kg, Table 3a) as a result of the significant decrease of PR mRNA in female VMN (Maerkel et al., in press). Acute estrogen challenge experiments in adult gonadectomized offspring revealed changes in estrogen sensitivity in adult, developmentally 4-MBC-exposed offspring also at brain level, with increased response of PR mRNA to estradiol in VMN



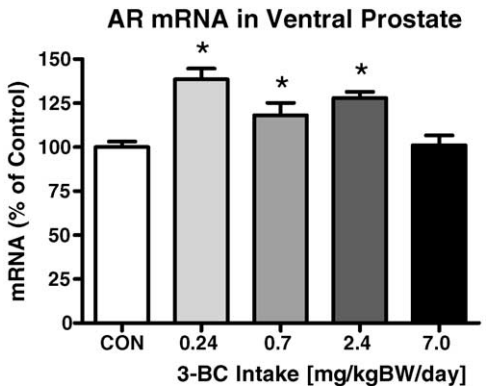


Fig. 2. Ventral prostate weight and androgen receptor (AR) mRNA level in F1 rat offspring at 12 weeks of age after developmental exposure to 3-benzylidene camphor (3-BC). Mean ± S.E.M., weight n = 12–19/group, AR mRNA n = 8–9/group.

of male and female offspring (Table 3a). Developmental 3-BC exposure likewise affects gene expression in central nervous system.

4. Conclusions

Our data reveal that a number of frequently used UV filters possess endocrine activity. Exposure to one of these, the estrogenic UV filter 4-MBC, during pre- and postnatal life was found to affect the development of the hypothalamo-pituitary-gonadal system of male and female rat offspring at central nervous system and peripheral level, and to result in changes in estrogen-regulated gene expression in reproductive organs and sexually dimorphic brain regions. The related camphor

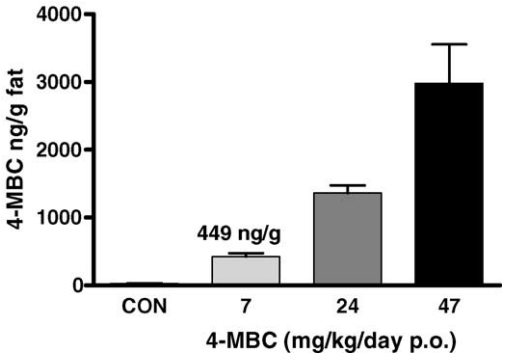


Fig. 3. Level of 4-methylbenzylidene camphor (4-MBC) in fat tissue of female F1 rats at the age of 12 weeks. Mean ± S.E.M., n = 3–9.

derivative 3-BC also exhibits developmental toxicity with partly different effect patterns. The toxicology of UV filters presents two facets, depending on exposure route: UV filters may reach the organism during usage of sunscreen products, but they also contribute to the mixture of endocrine active chemicals present in the environment. In the latter context, the combined effect of different chemicals is relevant. Classical toxicological endpoints and gene expression data of the developmental toxicity studies yielded the same minimum effective doses, i.e., 7 mg/kg/day for 4-MBC and 0.24 mg/kg/day for 3-BC. At 7 mg/kg 4-MBC, adipose tissue levels in adult F1 rat offspring, analyzed by GC–MS, were 449 ng/g lipid (Fig. 3). This level is close to the upper range of 4-MBC concentrations recently found in fish (roach and perch) of Swiss lakes (range 44–166 ng/g lipid, Balmer et al., 2004, cf. Nagtegaal et al., 1997). Tissue concentrations in humans have not been published so far. A human systemic exposure dose (SED) of 4-MBC has been estimated as 0.23 mg/kg body weight (SCCNFP, 1998). Such a dose would be only 1/3 of the present no observed adverse effect level (NOAEL) and 1/30 of the lowest observed adverse effect level (LOAEL) of 4-MBC. However, we plan to correlate rat adipose tissue levels with data from human milk in order to obtain a more solid basis for risk assessment.

From a general point of view, our data indicate that both, reproductive organs and central nervous system represent sensitive targets for developmental effects of endocrine active xenobiotics. Effect patterns differ in part between the estrogenic camphor derivatives, 3-BC and 4-MBC, and were again found to be different in parallel developmental studies with other en-

docrine active chemicals (genistein, diethylstilbestrol (unpublished observations), PCB and a polybrominated diphenylether (PBDE99) (Lichtensteiger et al., 2003). Thus, developmental effect patterns at the organ and molecular level appear to be difficult to predict from effects in simple acute in vitro or in vivo model systems, and should be carefully evaluated for different types of chemicals.

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