Les perturbateurs endocriniens (PE) dans les crèmes solaires représentent un risque pour l'adulte, mais aussi pour l'enfant.

L'attention a commencé à être attirée sur les risques liés aux PE dans les filtres anti-UV à la suite de la publication en 2001 d'un article de chercheurs de l'Institut de Pharmacologie et de Toxicologie de l'Université de Zürich (Schlumpf, 2001).

Cette étude montrait que 5 substances sur les 6 testées induisaient une réponse positive sur le test classique de détection de l'activité oestrogénique (sur cellules humaines de cancer du sein MCF-7) à des concentrations relativement faibles (de l'ordre du µM ou micromolaire). Ces substances, dont l'utilisation est très fréquente non seulement dans les crèmes solaires, mais aussi dans les produits anti-âge, les baumes à lèvres ou les crèmes hydratantes, étaient les suivantes : benzophénone-3 (BP3) appelée aussi oxybenzone, homosalate (HMS), 4-méthyl-benzylidène camphre (4-MBC), octyl-méthoxycinnamate (OMC), et octyl-diméthyl-PABA (OD-PABA). La 6^{e}, le butyl-méthoxydibenzoylméthane (B-MDM) était inactive.

Le 2nd test classique, dit utérotrophique (il mesure la croissance du poids de l'utérus chez le rongeur après intoxication par voie orale) était positif : nettement pour 2 de ces substances (4-MBC et OMC), plus faiblement pour BP3 et négatif pour les 3 autres. Le même phénomène était observé après application de 4-MBC sur la peau des rats.

Ces résultats ont été complétés par la même équipe, notamment en regardant les conséquences de l'exposition parentale sur la descendance (Schlumpf, 2004, 2008) :
- Effets oestrogéniques (Cellules MCF-7) pour 8 substances sur 9 : benzophénones 1, 2 et 3 (BP1, BP2, BP3), 3-BC, 4-MBC, HMS, OD-PABA, OMC. Aucun effet avec B-MDM.

- Effets anti-androgéniques pour 2/9 (Cellules MDA) : BP3 et HMS.
- Augmentation du poids de l'utérus pour 6 substances sur 9 : BP1, BP2, BP3, 3-BC, 4-MBC, OMC.
- 4-MBC et 3-BC retardent l'âge de la puberté chez les mâles et affectent le poids des organes de la reproduction dans la descendance mâle et femelle.

D’autres équipes ont confirmé ces résultats notamment en montrant des effets additifs (Henneweer, 2005), dont au moins une équipe travaillant pour l’industrie chimique (pour le 4-MBC, Tinwell, 2002).

La toute dernière étude de l’équipe suisse (Faass, 2009) est particulièrement intéressante. Deux substances (4-MBC et 3-BC) ont été testées. La première a été détectée dans le lait de la femme, ce qui conduit évidemment à s’interroger sur les risques pour l’enfant , consécutifs à cette exposition maternelle. Le protocole était le suivant : les 2 substances étaient administrées à des rats dans la nourriture

des parents avant accouplement, pendant la gestation et pendant la lactation, ainsi qu’à leurs descendants jusqu’à l’âge adulte.
Le comportement sexuel des rats femelles a été ensuite observé. Les 2 substances diminuent les comportements de préparation à l’accouplement et augmente le rejet des mâles. Le cycle menstruel n’était pas affecté par 4-MBC, mais par 3-BC. Des modifications de l’expression des gènes impliqués dans le comportement sexuel des femelles
ont pu être mises
en évidence. Ces effets surviennent à des
concentrations
proches de celles mesurées dans le lait maternel humain.

Deux études américaines récentes menées par un laboratoire des CDC (Centers for Disease Control) auprès de 2517 personnes âgées de plus de 6 ans montrent une imprégnation de quasiment toute la population américaine à une de ces substances, le BP3 : 96,8 % des échantillons d’urine contenaient en effet du BP3 (Calafat 2008). Les femmes étaient environ 2 fois plus exposées que les hommes. Plus curieusement, les Blancs (non Hispaniques) étaient 2 fois plus imprégnés que les Mexicains et 3 fois plus que les Noirs.

La même équipe a mis en évidence une imprégnation des prématurés (Calafat, 2009). Le taux était plus faible que celui des enfants mesurés par ailleurs, mais BP3 était cependant trouvé dans presque tous les échantillons. Cette étude montre par ailleurs une imprégnation plus élevée que la moyenne chez cette population pour d’autres perturbateurs endocriniens connus comme le BPA ou les phtalates.

La question se pose en effet de savoir quel est l’impact non seulement spécifiquement de ces substances présentes dans les crèmes solaires, mais du cocktail de perturbateurs endocriniens qui imprègnent aujourd’hui l’espèce humaine sur toute la planète.

Autre question montante qui en découle : ces substances sont retrouvées dans l'environnement, notamment dans l'eau, mais aussi dans les poissons. Avec quelles conséquences ?

Il n'y a pour l'instant pas de preuve épidémiologique chez l'homme, mais cette preuve semble difficile, si ce n'est impossible, à obtenir. D'une part, quasiment toute la population est imprégnée (nous disposons des données américaines, mais il serait souhaitable d'avoir des données françaises), d'autre part, cela supposerait d'attendre plusieurs décennies pour pouvoir évaluer les effets, comme par exemple le cancer du sein, ce que suggèrent les effets oestrogéniques de ces substances. C'est éthiquement inacceptable.

Le principe de précaution a justement été élaboré pour gérer ce type de situation en cas de « risques graves et irréversibles ». Des risques de cancers, des atteintes de la reproduction, des atteintes transgénérationnelles, la quasi-totalité de la population concernée… on est bien dans le cas de « risques graves et irréversibles ».

Il est donc préférable d'éviter d'exposer toute la population à ce type de substances. Les essais faits par l'ONG américaine Environmental Working Group montrent que des produits de substitution existent qui répondent à la fois aux exigences de protection contre les UV et d'absence d'impact sanitaire.

Résumés des articles cités :

Schlumpf M , Cotton B , Conscience M , Haller V , Steinmann B , Lichtensteiger W . In vitro and in vivo estrogenicity of UV screens.
Environ Health Perspect.
2001 Mar;109(3):239-44. Erratum in:

Environ Health Perspect. 2001 Nov;109(11):A517.
Comment in:
Environ Health Perspect. 2001 Nov;109(11):A517.

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Ultraviolet (UV) screens are increasingly used as a result of growing concern about UV radiation and skin cancer; they are also added to cosmetics and other products for light stability. Recent data on bioaccumulation in wildlife and humans point to a need for in-depth analyses of systemic toxicology, in particular with respect to reproduction and ontogeny. We examined six frequently used UVA and UVB screens for estrogenicity in vitro and in vivo. In MCF-7 breast cancer cells, five out of six chemicals, that is, benzophenone-3 (BP3), homosalate (HMS), 4-methyl-benzylidene camphor (4-MBC), octyl-methoxycinnamate (OMC), and octyl-dimethyl-PABA (OD-PABA), increased cell proliferation with median effective concentrations (EC(50)) values between 1.56 and 3.73 microM, whereas butyl-methoxydibenzoylmethane (B-MDM) was inactive. Further evidence for estrogenic activity was the induction of pS2 protein in MCF-7 cells and the blockade of the proliferative effect of 4-MBC by the estrogen antagonist ICI 182,780. In the uterotrophic assay using immature Long-Evans rats that received the chemicals for 4 days in powdered feed, uterine weight was dose-dependently increased by 4-MBC (ED(50)309mg/kg/day), OMC (ED(50) 935 mg/kg/day), and weakly by BP3 (active at 1,525 mg/kg/day). Three compounds were inactive by the oral route in the doses tested. Dermal application of 4-MBC to immature hairless (hr/hr) rats also increased uterine weight at concentrations of 5 and 7.5% in olive oil. Our findings indicate that UV screens should be tested for endocrine activity, in view of possible long-term effects in humans and wildlife.

Schlumpf M , **Schmid P** , **Durrer S** , **Conscience M** , **Maerkel K** , **Henseler M** , **Gruetter M** , **Herzog I** , **Reolon S** , **Ceccatelli R** , **Faass O** , **Stutz E** , **Jarry H** ,

Wuttke W
,
Lichtensteiger W
.

Endocrine activity and developmental toxicity of cosmetic UV filters--an update.

Toxicology.
2004 Dec 1;205(1-2):113-22.

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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, BP2, BP3, 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 16alpha125I-estradiol from human estrogen receptor (ER)beta, not ERalpha. 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).

Tinwell H , **Lefevre PA** , **Moffat GJ** , **Burns A** , **Odum J** , **Spurway TD** , **Orphanides G** , **Ashby J**
.

Confirmation of uterotrophic activity of 3-(4-methylbenzylidine)camphor in the immature rat.

Environ Health Perspect.
2002 May;110(5):533-6.

Syngenta Central Toxicology Laboratory, Cheshire, United Kingdom.

In this study we found that the ultraviolet sunscreen component 3-(4 methylbenzylidine)camphor (4MBC) is uterotrophic in immature rats when administered by either subcutaneous injection or oral gavage. These data confirm earlier reports of uterotrophic activity for this agent when administered to immature rats in the diet or by whole-body immersion; however, they are in contrast to negative unpublished immature rat uterotrophic assay results. Data also indicate that 4MBC binds to isolated rat uterine estrogen receptors and shows activity in a human estrogen receptor yeast transactivation assay; however, we considered both of these effects equivocal. In this study, we confirmed the original observation that 4MBC was active as a mitogen to MCF-7 breast cancer cells. We evaluated and discounted the possibility that the estrogenic activity of 4MBC is related to its bulky camphor group, which is of similar molecular dimensions to that of the weak estrogen kepone. Uncertainty remains regarding the mechanism of the uterotrophic activity of 4MBC.

Schlumpf M , **Durrer S** , **Faass O** , **Ehnes C** , **Fuetsch M** , **Gaille C** , **Henseler M** , **Hofkamp L** ,

Maerkel K
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Reolon S
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Timms B
,
Tresguerres JA
,
Lichtensteiger W
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Developmental toxicity of UV filters and environmental exposure: a review.

Int J Androl.
2008 Apr;31(2):144-51.

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Several ultraviolet (UV) filters exhibit estrogenic, some also anti-androgenic activity. They are present in waste water treatment plants, surface waters and biosphere including human milk, suggesting potential exposure during development. Developmental toxicity was studied in rats for the UV filters 4-methylbenzylidene camphor (4-MBC, 0.7, 7, 24, 47 mg/kg/day) and 3-benzylidene camphor (3-BC, 0.07, 0.24, 0.7, 2.4, 7 mg/kg/day) administered in chow to the

parent generation before mating, during pregnancy and lactation, and to the offspring until adulthood. Neonates exhibited enhanced prostate growth after 4-MBC and altered uterine gene expression after both chemicals. 4-MBC and 3-BC delayed male puberty and affected reproductive organ weights of adult offspring. Effects on the thyroid axis were also noted. Expression and oestrogen sensitivity of oestrogen-regulated genes and nuclear receptor coregulator levels were altered at mRNA and protein levels in adult uterus, prostate and brain regions involved in gonadal control and sexual behaviour. Female sexual behaviour was impaired by both filters; 3-benzylidene camphor caused irregular cycles. Classical endpoints exhibited lowest observed adverse effect levels (LOAELs) and no observed adverse effect levels (NOAELs) of 7/0.7 mg/kg for 4-MBC and 0.24/0.07 mg/kg for 3-BC. Molecular endpoints were affected by the lowest doses studied. Our data indicate that the potential risk posed by endocrine active UV filters warrants further investigations.

Heneweer M , Muusse M , van den Berg M , Sanderson JT . Additive estrogenic effects of mixtures of frequently used UV filters on pS2-gene transcription in MCF-7 cells. Toxicol Appl Pharmacol.
2005 Oct 15;208(2):170-7.

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In order to protect consumers from ultraviolet (UV) radiation and enhance light stability of the product, three to eight UV filters are usually added to consumer sunscreen products. High lipophilicity of the UV filters has been shown to cause bioaccumulation in fish and humans, leading to environmental levels of UV filters that are similar to those of PCBs and DDT. In this paper, estrogen-regulated pS2 gene transcription in the human mammary tumor cell line MCF-7 was used as a measure of estrogenicity of four individual UV filters. Since humans are exposed to more than one UV filter at a time, an equipotent binary mixture of 2-hydroxy-4-methoxy-benzophenone (BP3) and its metabolite 2,4-dihydroxy benzophenone (BP1), as well as an equipotent multi-component mixture of BP1, BP3, octyl methoxy cinnamate (OMC) and 3-(4-methylbenzylidene) camphor (4-MBC), were also evaluated for their ability to induce pS2 gene transcription in order to examine additivity. An estrogen receptor-mediated mechanism of action was expected for all UV filters. Therefore, our null-hypothesis was that combined estrogenic responses, measured as increased pS2 gene transcription in MCF-7 cells after exposure to mixtures of UV filters, are additive, according to a concentration-addition model. Not all UV filters produced a full concentration-response curve within the concentration range tested (100 nM-1 microM). Therefore, instead of using EC50 values for comparison, the concentration at which each compound caused a 50% increase of basal pS2 gene transcription was defined as the C50 value for that compound and used to calculate relative potencies. For comparison, the EC50 value of a compound is the concentration at which the compound elicits

an effect that is 50% of its maximal effect. Individual UV filters increased pS2 gene transcription concentration-dependently with C50 values of 0.12 microM, 0.5 microM, 1.9 microM, and 1.0 microM for BP1, BP3, 4-MBC and OMC, respectively. Estradiol (E2) had a C50 value of 4.8 pM. Experiments with equipotent mixtures all supported our null hypothesis that mixtures of UV filters act additively to activate the estrogen receptor (ER). In view of our results and observed plasma levels it cannot be excluded that daily exposure to sunscreen formulations may have estrogenic effects in humans.

Schlumpf M , **Durrer S** , **Faass O** , **Ehnes C** , **Fuetsch M** , **Gaille C** , **Henseler M** , **Hofkamp L** ,

Maerkel K
,
Reolon S
,
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impaired by both filters; 3-benzylidene camphor caused irregular cycles. Classical endpoints exhibited lowest observed adverse effect levels (LOAELs) and no observed adverse effect levels (NOAELs) of 7/0.7 mg/kg for 4-MBC and 0.24/0.07 mg/kg for 3-BC. Molecular endpoints were affected by the lowest doses studied. Our data indicate that the potential risk posed by endocrine active UV filters warrants further investigations.

Faass O , Schlumpf M , Reolon S , Henseler M , Maerkel K , Durrer S , Lichtensteiger W . Female sexual behavior, estrous cycle and gene expression in sexually dimorphic brain regions after pre- and postnatal exposure to endocrine active UV filters.
Neurotoxicology.
2009 Mar;30(2):249-60.

GREEN Tox and Institute of Anatomy, University of Zurich, Zurich, Switzerland.

The developing female brain represents a potential target for estrogenic environmental chemicals because it depends on estrogen but is exposed to low endogenous estrogen levels, thus facilitating competition by exogenous estrogen receptor (ER) agonists. We investigated effects of two estrogenic UV filters, 4-methylbenzylidene camphor (4-MBC) and 3-benzylidene camphor (3-BC). 4-MBC has been detected in human milk, indicating potential exposure of fetus and infant. The two chemicals were administered in chow to rats of the parent generation before mating, during pregnancy and lactation, and to their offspring until adulthood. Female sexual behavior was recorded on videotape in adult female offspring on proestrus evening at the beginning of the dark phase. 4-MBC (7 and 24mg/kg bw/day) and 3-BC (2.4 and 7mg/kg bw/day) reduced proceptive behavior (jump and ear wiggling) and receptive behavior (lordosis quotient), and increased rejection behavior towards the male. Estrous cycles were not affected by 4-MBC but disturbed by 3-BC. mRNAs encoding for genes involved in female sexual behavior, ERalpha, ERbeta, progesterone receptor (PR) and steroid receptor coactivator-1 (SRC-1), were measured by real-time RT-PCR in ventromedial hypothalamic nucleus (VMH) and medial preoptic area of adult male and female offspring (studied in diestrus) after pre- and postnatal exposure to 3-BC (0.24, 0.7, 2.4 and 7mg/kg bw/day). Gene expression was affected in a sex- and region-specific manner. PR mRNA in female VMH was reduced to male levels at dose levels of 2.4 and 7mg/kg bw/day 3-BC. Our data demonstrate that female sexual behavior represents a sensitive target of endocrine disrupters and point to an involvement of PR in VMH.

Calafat AM , Wong LY , Ye X , Reidy JA , Needham LL . Concentrations of the sunscreen agent benzophenone-3 in residents of the United States: National Health and Nutrition Examination Survey 2003--2004.

Environ Health Perspect.
2008 Jul;116(7):893-7.

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BACKGROUND: The capability of benzophenone-3 (BP3) to absorb and dissipate ultraviolet radiation facilitates its use as a sunscreen agent. BP3 has other uses in many consumer products (e.g., as fragrance and flavor enhancer, photoinitiator, ultraviolet curing agent, polymerization inhibitor). OBJECTIVES: Our goal was to assess exposure to BP3 in a representative sample of the U.S. general population > or = 6 years of age. METHODS: Using automated solid-phase extraction coupled to high-performance liquid chromatography-tandem mass spectrometry, we analyzed 2,517 urine samples collected as part of the 2003--2004 National Health and Nutrition Examination Survey. RESULTS: We detected BP3 in 96.8% of the samples. The geometric mean and 95th percentile concentrations were 22.9 microg/L (22.2 microg/g creatinine) and 1,040 microg/L (1,070 microg/g creatinine), respectively. Least-square geometric mean (LSGM) concentrations were significantly higher ($p < or = 0.04$) for females than for males, regardless of age. LSGM concentrations were significantly higher for non-Hispanic whites than for non-Hispanic blacks ($p < or = 0.01$), regardless of age. Females were more likely than males [adjusted odds ratio (OR) = 3.5; 95% confidence interval (95% CI), 1.9-6.5], and non-Hispanic whites were more likely than non-Hispanic blacks (adjusted OR = 6.8; 95% CI, 2.9-16.2) to have concentrations above the 95th percentile. CONCLUSIONS: Exposure to BP3 was prevalent in the general U.S. population during 2003--2004. Differences by sex and race/ethnicity probably reflect differences in use of personal care products containing BP3.

Calafat AM , Weuve J , Ye X , Jia LT , Hu H , Ringer S , Huttner K , Hauser R .
Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants.
Environ Health Perspect.
2009 Apr;117(4):639-44.

Comment in: Environ Health Perspect. 2009 Apr;117(4):A163.

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OBJECTIVE: We previously demonstrated that exposure to polyvinyl chloride plastic medical devices containing di(2-ethylhexyl) phthalate (DEHP) was associated with higher urinary concentrations of several DEHP metabolites in 54 premature infants in two neonatal intensive care units than in the general population. For 42 of these infants, we evaluated urinary concentrations of several phenols, including bisphenol A (BPA), in association with the use of the same medical devices. MEASUREMENTS: We measured the urinary concentrations of free and total (free plus conjugated) species of BPA, triclosan, benzophenone-3, methyl paraben, and propyl paraben. RESULTS: The percentage of BPA present as its conjugated species was > 90% in more than three-quarters of the premature infants. Intensity of use of products containing DEHP was strongly associated with BPA total concentrations but not with any other phenol. Adjusting for institution and sex, BPA total concentrations among infants in the group of high use of DEHP-containing products were 8.75 times as high as among infants in the low use group ($p < 0.0001$). Similarly, after adjusting for sex and DEHP-containing product use category, BPA total concentrations among infants in Institution A were 16.6 times as high as those among infants in Institution B ($p < 0.0001$). CONCLUSION: BPA geometric mean urinary concentration (30.3 microg/L) among premature infants undergoing intensive therapeutic medical interventions was one order of magnitude higher than that among the general population. Conjugated species were the primary urinary metabolites of BPA, suggesting that premature infants have some capacity to metabolize BPA. The differences in exposure to BPA by intensity of use of DEHP-containing medical products highlight the need for further studies to determine the specific source(s) of exposure to BPA.