In Vitro and in Vivo Estrogenicity of UV Screens

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Organic chemicals that absorb UVA (400–315 nm) or UVB (315–280 nm) radiation are added in concentrations up to 10% to sunscreen products for skin protection. Some of the compounds are also included in other cosmetics such as beauty creams, lipsticks, skin lotions, hair sprays, hair dyes, shampoos, and bubble baths for product stability and durability.

Because of growing public concern about skin damage by UV light, the use of UV screens is increasing, even though the benefit with respect to prevention of melanoma remains controversial. Like other cosmetics such as musk fragrances, these chemicals are highly lipophilic and therefore can be expected to bioaccumulate in the environment. In 1991 and 1993, six different UV screens were identified in fish of the Meerfelder Maar lake (Eifel, Germany) at total concentrations of 2 mg/kg lipid in perch (summer 1991) and 0.5 mg/kg lipid in roach (1993). Both fish species were contaminated with sunscreens, polychlorinated biphenyls and DDT at comparable levels. From these results it appeared that UV screens are relevant environmental contaminants.

Humans can be exposed to UV screens by dermal absorption (6–9) or through the food chain. The UV screen benzophenone-3 (Bp-3) and its metabolite 2,4-dihydroxybenzophenone have been detected in human urine from 4 hr after application of commercially available sunscreen products to the skin. Bp-3 has also been found to be readily absorbed from the gastrointestinal tract (11). Evidence for bioaccumulation in humans stems from analyses of human milk (12). In five out of six samples of human milk, Bp-3 and/or octyl methoxycinnamate were present in detectable amounts.

At present, the toxicologic classification of UV screens is rather heterogeneous; they are classified as over-the-counter drugs in the United States, cosmetics ingredients in the European Union, and either cosmetics or quasi-drug products in Japan. Acute and subchronic systemic toxicity of these compounds is considered to be rather low (7,14,15), although some problems have arisen with photoallergic reactions (16). No values of acceptable daily intake or maximal tolerated intake of UV screens have been defined. However, the bioaccumulation potential of these lipophilic chemicals does not appear to have been considered in earlier toxicologic long-term studies. The evidence of bioaccumulation in wildlife and humans raises the possibility of long-term exposure, including effects on reproduction and ontogeny. As a consequence, these compounds should be tested for endocrine activity.

We analyzed six frequently used UVA- or UVB-absorbing UV screens for estrogenic activity in vitro in MCF-7 breast cancer cells and in vivo in the immature rat uterotrophic assay. Estrogenic activity was demonstrated for five of six compounds in vitro and for three out of six compounds in vivo by the oral route. The orally most active compound also increased uterine weight following dermal application.

Materials and Methods

Chemicals

The UV screens Bp-3 (2-hydroxy-4-methoxybenzophenone, oxybenzone, Eusolex 4369); butyl methoxydibenzoylmethane (B-MDM, Eusolex 9020); homosalate (HMS, 2-hydroxybenzoic acid-3,3,5-trimethylcyclohexyl ester, Eusolex H M S); 3-(4-methylbenzylidene) camphor (4-M BC, Eusolex 6300); octyl-dimethyl-p-aminobenzoic acid (OD-PABA, Eusolex 6007); and octyl-methoxycinnamate (OMC, Eusolex 2292) were purchased from Merck (Dixtkon, Switzerland), 17β-Estradiol (E2) and 17α-ethinylestradiol were obtained from Ciba-Swiss (Lucerne, Switzerland), and ICI 182,780 (Astra-Zeneca) was purchased from ANAWA (Dübendorf, Switzerland).

In vitro Studies on MCF-7 Cells

The UV screens Bp-3, B-MDM, HMS, OMC, and 4-MBC were tested for estrogenic potency by the uterotrophic assay in vivo in the immature rat (hr/hr) (17). The estrogen antagonist ICI 182,780 was used to confirm the estrogenic activity of the UV screens. Bp-3 and 4-MBC at concentrations of 5 and 7.5 mg/kg in olive oil were found to be estrogenic in this assay (18).

In vitro estrogenic potency was determined in the MCF-7 human breast cancer cell line, as well as in the rat uterotrophic assay. The estrogen agonist 17β-Estradiol (E2) and 17α-ethinylestradiol were used as a positive control. The human breast cancer cell line MCF-7 was originally from the Michigan Cancer Foundation, Detroit, MI, USA. MCF-7 cells were cultured in Dulbecco’s modified Eagle Medium (DME) supplemented with 5% fetal bovine serum (FBS; Gibco, Life Technologies, Basel, Switzerland) in 5% CO2/95% air at 37°C under saturated
humidity in 50 mL Falcon flasks. Sex steroids were removed from the serum by charcoal dextran treatment (steroid hormone-free FBS (CD-FBS)) (17).

E-SCREEN. The E-SCREEN was performed according to Soto and co-workers (17,18). Briefly, M CF-7 cells were trypsinized, mechanically dissociated, and plated into 24-well plates (Costar; INTEGRA Biosciences, Wallisellen, Switzerland) at an initial concentration of 40,000 cells/well. Cells were allowed to attach in the seeding medium (DME supplemented with 5% FBS) for 24 hr. The seeding medium was then aspirated (DME supplemented with 5% FBS) for 24 hr. The seeding medium was then aspirated and replaced by the experimental medium containing phenol red-free DME with 10% CD-FBS. Cells were incubated with test compound (final concentrations 10\(^{-4}\)–10\(^{-7}\) M), E\(_2\) as positive control (10\(^{-8}\)–10\(^{-13}\) M), or chemical-free medium control (Table 1). For each concentration, 4 wells per plate were used. The number of independent in vitro experiments is given in Table 1 and in figures representing in vitro data. Each experiment was accompanied by a positive control (E\(_2\)). Concentrations of stock solutions in absolute ethanol were 2 \times 10^{-3} M for E\(_2\) and 2 \times 10^{-2} M for UV screens; final concentrations of ethanol in culture medium were between 1.0% and 0.001% (v/v) with test compounds, and were ≤ 0.0005% (v/v) with E\(_2\). No difference in the cell proliferation rate was observed in control experiments with chemical-free medium or medium with 1.0% ethanol. Therefore, we used chemical-free medium as a control. We tested antagonism by the pure antiestrogen ICI 182,780 (Provirin Klìba AG, Kaiseraugst, Switzerland) and water ad libitum. All experiments were performed on offspring of timed-pregnant rats. Receptive females were mated with a male between 1600 and 1900 hr. Sperm-positive females were housed in groups of two to three and separated 1 day before parturition. We defined the stage 24 hr after onset of the 3-hr mating period as gestational day (GD) 1 and the day of birth as postnatal day (PN) 1 (GD 23).

Peroral administration of test chemicals. From PN 16, the pups and their dam were habituated to powdered chow (chow 3430, Provirin Klìba), which continued after weaning at PN 20. Beginning on PN 21, female pups received chow 3430 containing one of several concentrations of test compound for 4 days, until 1200 hr on PN 25. For each experiment, chemicals were dissolved either in acetone or in 99% ethanol and added to powdered chow 3430. The mixture was prepared at least 48 hr before the experiment to allow for complete evaporation of the solvent. Evaporation was assisted by continuous stirring. We used ethinylestradiol (0.3–10 \(\mu\)g/kg) as a positive control. Vehicle controls were normal chow 3430. To limit the number of experimental animals, we adjusted the size of the various treatment groups according to statistical needs. We used the minimum group sample size of the three UV screens that we determined were inactive.

To avoid stress to the immature pups, we housed the animals in groups of four to six. We recorded body weight at the beginning and at the end of the treatment period. Animals from different litters were randomly assigned to the various treatment groups to give similar mean body weights to the various treatment groups at the onset of treatment, with no more than 15% deviation of individual animals from the mean. Mean body weights of experimental groups were in the same range as that of the controls (Table 1).

Table 1. Effect of UV screens and E\(_2\) on M CF-7 cell proliferation in vitro.

| Compound | Concentration (M) | Cells/well |
|----------|------------------|------------|
| E\(_2\)   | 0                | 44,501 ± 5,079 (13) |
| E\(_2\)   | 1 \times 10^{-1} | 41,393 ± 12,069 (4) |
| E\(_2\)   | 5 \times 10^{-1} | 61,563 ± 2,909 (6) |
| E\(_2\)   | 1 \times 10^{-2} | 333,970 ± 51,026 (11) |
| E\(_2\)   | 1 \times 10^{-3} | 743,296 ± 68,655 (11) |
| E\(_2\)   | 1 \times 10^{-4} | 677,115 ± 91,301 (10) |
| E\(_2\)   | 1 \times 10^{-5} | 655,969 ± 77,928 (10) |
| E\(_2\)   | 1 \times 10^{-6} | 623,608 ± 72,292 (11) |
| 4-MBC    | 0                | 49,028 ± 15,824 (6) |
| 4-MBC    | 1 \times 10^{-7} | 132,292 ± 33,478 (6) |
| 4-MBC    | 1 \times 10^{-6} | 147,396 ± 46,267 (6) |
| 4-MBC    | 5 \times 10^{-6} | 583,269 ± 51,178 (6) |
| 4-MBC    | 1 \times 10^{-5} | 66,597 ± 66,740 (6) |
| 4-MBC    | 5 \times 10^{-5} | 300,157 ± 68,896 (6) |
| OM C     | 0                | 55,504 ± 13,373 (6) |
| OM C     | 1 \times 10^{-7} | 147,033 ± 25,657 (6) |
| OM C     | 1 \times 10^{-6} | 229,688 ± 65,150 (6) |
| OM C     | 5 \times 10^{-6} | 594,809 ± 74,438 (6) |
| OM C     | 1 \times 10^{-5} | 566,945 ± 68,285 (6) |
| OM C     | 5 \times 10^{-5} | 215,983 ± 58,542 (6) |
| Bp-3     | 0                | 40,292 ± 2,422 (5) |
| Bp-3     | 1 \times 10^{-7} | 99,605 ± 28,489 (5) |
| Bp-3     | 1 \times 10^{-6} | 71,438 ± 29,796 (5) |
| Bp-3     | 5 \times 10^{-6} | 448,750 ± 78,557 (5) |
| Bp-3     | 1 \times 10^{-5} | 704,750 ± 53,108 (5) |
| Bp-3     | 5 \times 10^{-5} | 680,354 ± 63,914 (5) |
| HMS      | 0                | 96,292 ± 15,512 (5) |
| HMS      | 1 \times 10^{-7} | 155,771 ± 20,505 (5) |
| HMS      | 1 \times 10^{-6} | 279,833 ± 22,404 (5) |
| HMS      | 5 \times 10^{-6} | 573,042 ± 50,308 (5) |
| HMS      | 1 \times 10^{-5} | 652,917 ± 35,943 (5) |
| HMS      | 5 \times 10^{-5} | 586,479 ± 14,416 (5) |
| OD-PABA  | 0                | 47,726 ± 14,068 (6) |
| OD-PABA  | 1 \times 10^{-7} | 113,229 ± 24,761 (6) |
| OD-PABA  | 1 \times 10^{-6} | 76,719 ± 6,740 (6) |
| OD-PABA  | 5 \times 10^{-6} | 290,181 ± 59,554 (6) |
| OD-PABA  | 1 \times 10^{-5} | 435,827 ± 35,692 (6) |
| OD-PABA  | 5 \times 10^{-5} | 326,021 ± 63,239 (6) |
| B-MDM    | 0                | 28,125 ± 5,381 (5) |
| B-MDM    | 1 \times 10^{-7} | 99,104 ± 35,589 (5) |
| B-MDM    | 1 \times 10^{-6} | 29,833 ± 6,559 (5) |
| B-MDM    | 5 \times 10^{-6} | 73,250 ± 46,864 (5) |
| B-MDM    | 1 \times 10^{-5} | 120,846 ± 79,925 (5) |
| B-MDM    | 5 \times 10^{-5} | 54,297 ± 19,109 (5) |

Values shown are mean ± SEM (number of independent experiments). * p < 0.05, ** p < 0.01, *** p < 0.001 JANova plus Bonferroni pairwise comparisons) as compared to control.
of the condition is indicated by an SD/mean ratio of uterine weights of 5.6% in controls and 9.8% in treated groups.

At the end of the treatment period, pups were decapitated under light ether anesthesia. The uterus was excised, trimmed free of fat and connective tissues, and blotted with sterile gauze to remove adherent fluid. The uterus was cut just above the junction between the cervix and vagina and at the top of the uterine horns. It was then weighed (wet weight) and either frozen in liquid nitrogen or fixed in buffered 4% formaldehyde for further analysis.

**Dermal application of test chemicals.** We studied possible effects of dermal application of UV screens in immature females of the Rat Nu (hairless) strain (Ico: OFA hr/hr). Parent animals were obtained from IFFA CREDO (Labresle, France) and kept under the same conditions as the Long-Evans rat colony (see above). One hr+/hr+ male was caged with three adult hr+/hr+ females. Pregnant dams were identified by weight gain, and the date of parturition (PN 1) was registered. Because of difficulties of the lactating hr+/hr+ dams to produce sufficient milk, the dam was replaced on PN 2 by a lactating Long-Evans dam. Pups were weaned at PN 20.

Female rat pups were treated on 6 consecutive days, from PN 21 to PN 26. 4-M BC [2.5%, 5.0%, 7.5% (w/w) in olive oil] or olive oil (vehicle control) was applied twice daily at an interval of 3–4 hr. At 30°C ambient temperature, the animal was gently held by the neck and immersed up to its shoulders into a glass beaker containing olive oil with 4-M BC or pure olive oil for 15 sec. The pup was then transferred into a plastic box (one animal per box), where it remained on a paper towel for 30 min. During the 30-min period, an additional amount of the solution was applied twice onto the back of the animal with a soft brush. After 30 min, the skin appeared dry; the animal was transferred onto a clean paper towel to remove remaining solution and then returned to its home cage. We used separate plastic boxes for 4-M BC-treated pups and controls. The animals were continuously observed; they did not lick their skin, but remained in a quiet position. On PN 27, pups were weighed and decapitated under light ether anesthesia. The uterus was removed and weighed as described above. The treatment group was unknown to the person dissecting the uterus.

In the absence of toxicokinetic in vivo data, it is not possible to exactly determine the amount of 4-M BC taken up by dermal application. However, we determined the amount of olive oil applied during one treatment by weighing the animal before and after each manipulation. The average amount of oil retained after 15 sec immersion was 1.35 ± 0.13 g and the additional amount applied by the brush was 1.4 ± 0.08 g. Thus, the total amount of oil was 2.75 g/treatment or 5.5 g/day, yielding total amounts of 4-M BC applied to the skin per day of 137.5, 275, and 412.5 mg for 2.5, 5.0, and 7.5% 4-M BC concentrations, respectively. According to in vitro data (8), the penetration of 4-M BC through hairless rat skin is 0.6% from oily gels or 0.4% from a water in oil (W/O) emulsion. Assuming 0.6% penetration, the dose absorbed from a 5% 4-M BC solution in oil can be tentatively calculated as 37 mg/kg/day based on mean body weight of the 5% group.

**Data Analysis.**

**In vitro studies.** We calculated cell counts per well from optical density as described above. In every experiment, we analyzed each concentration in quadruplicate. From these values, we calculated the mean cell count of a given concentration of chemical or of chemical-free medium for each experiment. Cell counts from different independent experiments were compared using analysis of variance (ANOVA) followed by Bonferroni pairwise comparisons (SYSTAT software; SYSTAT Intelligent Software, Evanston, IL, USA). The proliferative effect (PE) of a compound was defined as (maximal mean cell count obtained with the test chemical)/(mean cell count in the chemical-free medium), the relative proliferative effect (RPE) in relation to that of E₂ as (PE of test compound – 1)/[(PE of E₂ – 1) × 100]. For comparison with uterotrophic data, we expressed the increase in cell number as percentage of E₂ [(cell number with test compound – control)/(cell number with E₂ – control) × 100]. We calculated the median effective concentration (EC₅₀) values from the ascending part of the concentration–response curve using Graph Pad Prism2 software (Graph Pad Software, Inc., San Diego, CA, USA). For pS2 protein, treated groups and the chemical-free medium group were compared by ANOVA followed by Bonferroni pairwise comparisons (SYSTAT).

**In Vivo Studies.**

Uterine weights of individual animals from different dose groups and vehicle controls were compared by ANOVA followed by Bonferroni pairwise comparisons. We calculated ED₅₀ values using Prism2.

**Table 2. Comparison of in vitro and in vivo activity of UV screens and steroidal estrogens.**

| Compound   | PE⁹ | RPE⁹ | Maximal cell count increase (% of E₂) | EC₅₀ | Increase of uterine weight over control | Maximal weight increase (% of E₂) | ED₅₀ |
|------------|-----|------|--------------------------------------|------|----------------------------------------|-----------------------------------|------|
| E₂         | 16.70 | 100  | 100                                  | 1.22 μM | 4.08                                   | 100                              | 0.818 μg/kg/day |
| EE₂        | 11.74 | 105.0 | 95.09                                | 3.73 μM | 1.23                                   | 7.60                             | 1,000–1,500 μg/kg/day |
| Bp-3       | 13.49 | 79.54 | 87.66                                | 3.02 μM | 2.09                                   | 35.51                            | 309 μg/kg/day   |
| 4-MBC      | 10.72 | 61.90 | 77.18                                | 2.37 μM | 1.68                                   | 22.21                            | 934 μg/kg/day   |
| OM C       | 9.13  | 51.77 | 55.54                                | 2.63 μM | 1.04                                   | 1.15                             | Inactive       |
| OD-PABA    | 6.78  | 36.81 | 79.65                                | 1.56 μM | 1.12                                   | 3.79                             | Inactive       |
| H M S      | 4.30  | 21.01 | 13.27                                | 1.06   | 1.06                                   | 2.01                             | Inactive       |

⁹Proliferative effect over control; PE = [(maximal cell count of experimental group)/cell count of control].
**Results**

**Effect of UV Screens on MCF-7 Cells in Vitro**

**MCF-7 cell proliferation.** Cell proliferation was dose-dependently increased by all UV screens tested except for B-MDM, with a bell-shaped dose–response curve (Tables 1 and 2, Figure 2). The effective concentration range (1–50 µM) and the maximum effect concentration (at around 10 µM) was similar for the various compounds. In vitro EC50 values of UV screens range between 1.56 µM (HMS) and 3.73 µM (Bp-3) (Table 2). According to their maximum effects on cell proliferation in relation to E2, 4-MBC, OMC, OD-PABA, and HMS acted as partial agonists, whereas the maximum activity of Bp-3 reached the level of E2. The proliferative agonists, whereas the maximum activity of OMC, OD-PABA, and HMS acted as partial agonists (data not shown) and in the range of the respective treatment groups were similar (mean ± SD of 38.0 ± 4.5 g at PN 21 and 48.8 ± 3.9 g at PN 25).

**Dermal application of 4-MBC.** Following dermal application of 4-MBC in olive oil twice daily for 6 days, immature rats of the hr/hr (hairless) strain exhibited a dose-dependent increase in uterine weight, with a significant increase above control induced by 5% and 7.5% 4-MBC (Figure 5). The mean uterine weight of the 5% 4-MBC group was also significantly higher than that of the 2.5 or 7.5% groups, yielding a bell-shaped dose-response curve. The control uterine weight of this strain appeared to be slightly lower than that of Long-Evans rats, even though the animals were 2 days older. Mean body weights (± SD) of the various groups were similar at the beginning (control), 34.78 ± 3.15; 2.5% 4-MBC, 32.54 ± 1.40; 5% 4-MBC, 34.44 ± 4.10; 7.5% 4-MBC, 34.51 ± 1.92) and at the end of the treatment period (control), 52.52 ± 7.41; 2.5% 4-MBC, 43.63 ± 0.81; 5% 4-MBC, 55.99 ± 5.38; 7.5% 4-MBC, 51.11 ± 5.07.

**Discussion**

The present study demonstrates in vitro and in vivo estrogenic activity for a number of UV screens with different chemical structures. The compounds tested are frequently used in powdered feed for 4 days, three of the five compounds with in vitro activity, HMS and OD-PABA, as well as B-MDM, were inactive in vivo at the doses tested. Mammalian body weights of the various treatment groups were similar (mean ± SD of 38.0 ± 4.5 g at PN 21 and 48.8 ± 3.9 g at PN 25). The correlation between the increase in uterine weight and pS2 induction are different for the two parameters.

**Effect of UV Screens on the Immature Rat Uterus in Vivo**

**Peroral administration.** After administration in powdered feed for 4 days, three of the five chemicals active in vitro and the positive control ethinylestradiol elicited dose-dependent increases in uterine weight of immature Long-Evans rats (Table 3, Figure 2). The rank order of potency, 4-MBC > OMC > Bp-3, differed from the one observed in vitro; 4-MBC exhibited a significant increase in uterine weight at a dose of 119 mg/kg/day and an ED50 of 309 mg/kg/day (Tables 2, 3). Two of the compounds with in vitro activity, HMS and OD-PABA, as well as B-MDM, were inactive in vivo at the doses tested. Mammalian body weights of the various treatment groups were similar (data not shown) and in the range of the vehicle control group (mean ± SD of 38.0 ± 4.5 g at PN 21 and 48.8 ± 3.9 g at PN 25).

**Figure 2.** Dose–response relationship of estrogenic activity of different UV screens. (A) In vitro effect of UV screens on MCF-7 cell proliferation (cell number/well) as a percentage of the maximum effect of E2. (B) Effect of oral UV screens on immature rat uterine weight as a percentage of the maximum effect of ethinylestradiol (EE2).
Three UV screens, 4-MBC, OMC, and Bp-3, were active by the oral route in an acute mammalian in vivo model for estrogenicity (21), eliciting dose-dependent increases of uterine weight in immature rats. Differences between individual UV screens were more pronounced than in vitro. 4-MBC was most active, with a significant increase in uterine weight at 119 mg/kg/day and an ED$_{50}$ of 309 mg/kg/day; it also had the greatest maximal effect (Table 2). In contrast, Bp-3 displayed only weak activity in vivo. This compound has recently been reported to be inactive in the uterotrophic assay (25). The dose level used was also ineffective in our investigation; thus, the two data sets are in agreement. The in vitro (proliferation) and in vivo dose–response curves of 4-MBC and OMC suggest that they are partial agonists. Bp-3 is difficult to judge because it reached the level of the full agonist, E$_2$, in vitro, whereas the in vivo dose–response curve is incomplete.

Weak binding to estrogen receptors has been reported for unsubstituted benzophenone (26). One of the main metabolites of Bp-3, 2,4-dihydroxybenzophenone, binds to estrogen receptors with micromolar affinity, in contrast to its parent compound (27). This metabolite was detected in human urine after dermal application of a commercial sunscreen product (10). O-dealkylation also appears to be the major metabolic pathway of Bp-3 in rats (28). However, the relative roles of parent compounds and metabolites for in vivo estrogenic activities of the various UV screens remain to be clarified.

A comparison of in vitro and in vivo data indicates that the in vitro assay was useful for identifying estrogenic activity, but was of limited predictive value for the mammalian in vivo situation. Although all three chemicals that exhibited in vivo activity were strongly active in vitro, two compounds with high or moderate activity in vitro, HMS and OD-PABA, were completely inactive in the uterotrophic assay at the doses tested. The rank orders of activity also differed between in vitro and in vivo experiments. This may have resulted from pharmacodynamic and/or pharmacokinetic differences. A precise quantitative comparison of in vitro and in vivo effects is not possible because different tissues served as end points and different estrogens were used as positive controls. The differences between in vitro and in vivo data support the need for in vivo testing of chemicals before identification of endocrine activity in vitro.

The investigated chemicals are diverse in structure, but they share a common use as UV A or UV B screens and they have potential for bioaccumulation. Four of the UV screens with estrogenic activity, 4-MBC, Bp-3, HMS, and OMC, have been detected in fish (5), and so far two compounds, Bp-3 and OMC, have been detected in human milk (12). The total concentration of estrogenic UV screens in fish, where a larger data set is available, ranged between 1.6 µM and 7.8 µM in fat, or between 0.02 and 0.2 µM in whole fish (roach and perch, respectively). UV screens thus may contribute to the total genicity in wildlife and humans. The effective dose range of oral 4-MBC, OMC, and Bp-3 in the rat uterotrophic assay (119 mg/kg/day for 4-MBC to 1,500 mg/kg/day for Bp-3) compares with daily oral doses of bisphenol A (400 mg/kg) (29), methoxychlor (100–500 mg/kg), nonylphenol (190–1,000 mg/kg), and o,p’-DDE (1,000 mg/kg) (21,30) that increase rodent uterine weight. Uterine epithelial growth factor receptor was induced by 500 mg/kg/day methoxychlor (31). In one study on bisphenol A, Gould et al. (32) were unable to detect changes in uterine weight, but they reported an increase in peroxidase activity at 100 mg/kg/day and increased progesterone receptor levels at lower doses. These doses cannot be compared with actual exposure levels, as it is generally agreed that the acute high-dose rodent model cannot serve as a basis for risk assessment, but rather for identification of in vivo activity. Thus, bisphenol A has been found to disturb developmental processes at doses that are several orders of magnitude lower (2–50 µg/kg) (33,34).

As UV screens, the chemicals tested in this study present two different toxicologic aspects: On one hand, they may play an ecotoxicologic role in wildlife and humans, probably resulting mainly from intake via the food chain. On the other hand, they may also be transferentially active in humans when they are used as sunscreens. We observed an increase in uterine weight after dermal application of

![Figure 4. Effect of UV screens 4-MBC (10 µM), HMS (50 µM), Bp3 (10 µM), OMC (10 µM), OD-PABA (10 µM), B-MDM (10 µM), and E$_2$ (10 µM) on pS2 protein secretion by MCF-7 cells. Bars indicate mean pS2 protein concentration in culture medium (ppg/ml) ± SEM; numbers inside the bars indicate the number of independent experiments). *p < 0.05, **p < 0.002 (ANOVA followed by Bonferroni pairwise comparisons) as compared to controls that received chemical-free medium.](image)

![Figure 5. Effect of dermal application of 4-MBC (2.5, 5.0, or 7.5 % in olive oil, twice daily for 6 days) on uterine weight of immature hairless (hr+/hr+) rats. Controls received vehicle (olive oil). Bars indicate mean ± SD; numbers inside the bars indicate the number of animals. *5% 4-MBC different from 2.5%, p < 0.005; different from 7.5%, p < 0.001; †p < 0.001; ††p < 0.025 as compared to controls (ANOVA followed by Bonferroni pairwise comparisons).](image)
4-M BC in olive oil to immature hairless rats at concentrations allowed in sunscreen products. The dose–response curve of uterine weight was bell-shaped, suggesting more complex interactions. With 5% 4-M BC, the increase (159% of control) corresponded to the increase (154% of control) produced by an oral dose of 337 mg/kg/day, which is close to the oral ED₅₀. 4-M BC exhibits significant penetration through skin of hairless rats from a 6% solution in either a W/O emulsion or oily gels (8). BP-3 is also dermally absorbed in rats (35). Evidence for absorption by human skin has been presented for 4-M BC, BP-3, and OD-PABA (6,7,9,10); 4-M BC also penetrates a follicle-anine membrane, a model for human skin (8). Skin penetration may vary between compounds, as indicated by lower penetration of OMC as compared to BP-3 (3,36), and appears to be influenced by the formulation (8,36). Such kinetic differences may be of importance from a toxicologic point of view, but present knowledge is too incomplete to provide a picture of the general human exposure to UV screens.

Table 3. Effect of oral UV screens and ethynylestradiol on uterine weight of immature rats.

| Dose          | Uterine weight (mg) |
|---------------|---------------------|
| Ethynylestradiol (μg/kg/day) |                     |
| 0.015         | 29.15 ± 3.59 (6)    |
| 0.342         | 37.02 ± 1.05 (5)*   |
| 0.780         | 61.82 ± 5.00 (5)*   |
| 0.856         | 72.80 ± 5.77 (5)*   |
| 1.648         | 102.86 ± 13.09 (5)* |
| 3.231         | 100.95 ± 32.8 (5)   |
| 4-M BC (mg/kg/day) |                   |
| 56            | 27.25 ± 1.72 (10)  |
| 109           | 32.43 ± 3.61 (13)* |
| 211           | 35.24 ± 5.84 (19)  |
| 337           | 38.78 ± 3.66 (18)**|
| 402           | 45.22 ± 8.23 (9)*  |
| 1,980         | 52.80 ± 11.8 (4)**  |
| OMC (mg/kg/day) |                   |
| 268           | 24.95 ± 2.50 (10)  |
| 522           | 26.81 ± 1.64 (13)  |
| 1,035         | 35.46 ± 8.47 (10)**|
| 1,518         | 39.59 ± 7.58 (7)**  |
| 2,667         | 42.48 ± 12.5 (5)**  |
| BP-3 (mg/kg/day) |                   |
| 513           | 26.84 ± 1.87 (5)   |
| 537           | 26.94 ± 2.26 (5)   |
| 1,525         | 31.4 ± 3.3 (3)*    |
| HM S (mg/kg/day) |                  |
| 491           | 28.18 ± 1.64 (6)   |
| 892           | 23.36 ± 10.6 (5)   |
| OD-RABA (mg/kg/day) |                |
| 596           | 26.0 ± 0.95 (4)    |
| 761           | 24.75 ± 1.29 (6)   |
| 1,419         | 26.13 ± 3.10 (6)   |
| B-M DM (mg/kg/day) |                |
| 421           | 26.80 ± 1.08 (6)   |
| 636           | 26.05 ± 0.97 (6)   |
| Vehicle control | 25.24 ± 1.41 (28)  |

Values shown are mean ± SD (number of rats). *p < 0.0001, †p < 0.002 (ANOVA plus Bonferroni pairwise comparisons) as compared to controls.

**Conclusions**

Our investigation revealed that several frequently used UV screens possess estrogenic activity in vitro and in vivo, in the range of other known xenoestrogens. With the exception of some benzophenones, these chemicals do not appear to have been considered as potential environmental endocrine disruptors (37). Considering the widespread use of UV screens, we suggest that toxicokinetics, in particular skin penetration, and systemic toxicity of these chemicals should be investigated more extensively. In view of possible long-term effects, screening for estrogenic activity seems important. From our data and from observations in other fields (see above), it appears that there is a need to reconsider the potential benefits of extensive UV screen use both from a medical and an ecological perspective.

**References and Notes**

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