# Sunscreens – potential oestrogenic activity

## Abstract

*An article published in March 2001 (Schlumpf, et al: In-vitro and In-vivo Estrogenicity of UV Screens; Environmental Health Perspectives; vol. 109[3]; 239-244; March 2001) described a series of in-vitro and in-vivo assays that suggest some sunscreens possess oestrogenic activity. This paper discusses the results of the study and their relevance to the Australian situation, summarises international reaction at the time of writing and concludes that further action is not required at the present time.*

## Summary of the study

It was stated that UV screens were increasingly used as a result of growing concern about UV radiation and skin cancer, and this increase in use could lead to increases in environmental contamination (exposure in the food chain). This proposal was supported by the finding that measurable levels (up to 2 ppm) of UV screens had been identified in fish from a lake in Germany (lake Meerfelder Maar). It was noted that these fish were also contaminated with PCB's and DDT at comparable levels to the sunscreen compounds.

The general thrust of the article revolved around possible bio-accumulation of agents in wildlife and humans (mentioned above), and how these agents require closer examination for potential adverse biological activity. The primary focus of this article was to investigate possible oestrogenic activity, as demonstrated in a series of *in vitro* and *in vivo* assays, of 6 frequently used sunscreen agents. Sunscreens agents studied were benzophenone-3 (Bp-3), homosalate (HMS), 4-methyl-benzylidene camphor (4-MBC), octyl-methoxycinnamate (OMC), octyl-dimethyl-ρ-aminobenzoic acid (OD-PABA) and butyl methoxydibenzoylmethane (B-MDM).

## Bioassays for oestrogenic activity

a) MCF-7 human breast cancer cell assay (*in vitro*)

Assessment of oestrogenic activity *in vitro* involved a cell line of MCF-7 human breast cancer cells that were cultured following appropriate checks for mycoplasma status (negative) and cleansed of sex steroid contamination. Seeded growing cultures (in Dulbecco's modified medium) were incubated with varying concentrations of each sunscreen for testing, as well as positive (17β-estradiol [E2]) and negative (chemical-free medium) controls. At least 5 independent experiments were carried out for each concentration point of all agents and controls. The results were expressed as number of cells/well and statistical analysis was used to identify significant increases in cell numbers.

There was no significant difference in cell proliferation rate observed for controls across the experiments testing E2 and UV screens. Effects of UV screens and E2 on MCF-7 cell proliferation *in vitro* were subject to large variability. E2 significantly increased cell numbers from $1x10^{-12}$ M, while the majority of UV screens tested significantly increased cell numbers from $5x10^{-6}$ M (except for HMS, which was effective from $1x10^{-6}$ M). There was no significant effect of B-MDM on cell proliferation across a concentration range of $5x10^{-5}$ to

$1x10^{-7}$ M. Additional analyses to determine EC50 values for the test agents on MCF-7 cell proliferation produced the following data:

| MCF-7 cell proliferation data | | |
|---|---|---|
| Compound | Maximal cell count increase (% of E2) | EC50 |
| E2 | 100 | 1.22 pM |
| Bp-3 | 95.09 | 3.73 μM |
| 4-MBC | 87.66 | 3.02 μM |
| OMC | 77.18 | 2.37 μM |
| OD-PABA | 55.54 | 2.63 μM |
| HMS | 79.65 | 1.56 μM |
| B-MDM | 13.27 | inactive |

The order of potency was HMS>OMC>OD-PABA>4-MBC>Bp-3, with B-MDM inactive. The data indicated that the UV screens tested were at least $10^6$ times less active than the reference compound 17β-estradiol as an oestrogenic agent against MCF-7 human breast cancer cells *in vitro*. It was stated that the 5 UV screens showing oestrogenic activity did so in a dose-dependent manner; this statement does not appear to be supported by the data without eliminating higher concentrations (possibly assuming cell toxicity at higher concentrations), which cause lower cell growth and presumably lower oestrogenic activity. Furthermore, that the oestrogenic activity of the 5 UV screens was said to be in the range of other industrial chemicals identified as environmental oestrogens. Agents such as β-endosulfan, toxaphene, DDT and nonylphenol caused maximum proliferative (displayed oestrogenic activity) from a concentration of 1 μM, which indicates that they are probably more potent than the most active (ED50 range 1.56-3.73 μM) UV screen tested.

Note: The *in vitro* assay was known as an E-screen (according to Soto et al, 1995), which is based on oestrogens being defined by their ability to induce the proliferation of cells of the female genital tract. It was stated that the wide chemical diversity of oestrogenic compounds precludes an accurate prediction of oestrogenic activity on the basis of chemical structure. Rodent bioassays were said to be unsuitable for large scale screening of chemicals because of cost, complexity and ethical reasons.

b) Rodent uterotrophic assay (*in vivo*)

Groups of laboratory bred Long Evans rats were used in this study. The animals used in the study were females that had been born 21 days prior to dietary treatment with the test materials began. Study animals were fed a diet containing one of several concentrations of the test compounds for 4 days (until post-natal day 25). Included in the study were positive (ethinylestradiol) and vehicle (diet only) controls. At the end of the treatment period the animals were sacrificed, which was followed by their uterus being excised, trimmed free of fat and connective tissue, and weighed.

An additional section of this study examined the effect of topically applied 4-MBC (2.5%, 5.0% and 7.5% w/w in olive oil) as a uterotrophic agent on immature female Nu (hairless) strain Long Evans rats. The animals were treated on 6 consecutive days (post-natal days 21-26) by immersing (gently held at neck and immersed to shoulders) them twice daily in the oil for 15 second, with 3-4 hours between applications. In addition, each time the animals were

removed from the oil they were placed on a paper towel and had the oil (with test agents) gently rubbed into their back with a soft brush for 30 minutes. These animals were processed as above prior to the assessment of the uterotropic effect. There were no toxicokinetic data generated for the topical application of 4-MBC, therefore an exact systemic exposure for this process cannot be determined. An estimate based on *in vitro* data was presented that took into account penetration of 4-MBC through hairless rat skin of 0.6% from oily gels or 0.4% from a water in oil emulsion. It was stated that an estimation of 37 mg/kg/day would have occurred for the 5% group. The process involved in reaching this value of 37 mg/kg/day was not clear.

Following oral administration of the 6 UV screens, 4-MBC, OMC and Bp-3 were found to cause a significant increase in uterine weight with ED50 values of 309, 934 and 1000-1500 mg/kg/day, respectively. The order of potency was 4-MBC>OMC>Bp-3, with the other UV filters inactive. The other UV screens (OD-PABA, HMS and B-MDM) tested did not have a significant effect on uterine weight. It should be noted that the potency of the positive control (ethinylestradiol) was $10^5$ to $10^6$ greater than the UV filters inducing a positive response. Dermal application of 4-MBC resulted in a significant increase in uterine weight at the 5% and 7.5% dose, but this effect was not dose-related. The uterine weights for the control, 2.5%, 5% and 7.5% groups were 24, 28, 36 and 29 mg, respectively (taken from bar histogram). The size of the groups varied markedly, with an n of 11, 4, ? (not clear, taken from histogram) and 8 for the control, 2.5%, 5% and 7.5% groups, respectively.

Comments on the bioassay studies

An inconsistency in the results would appear to have occurred in the dose leading to an uterotrophic effect for the oral and dermal routes. Increased uterine weight was seen at 309 mg/kg/day 4-MBC, which was the lowest oral dose this change was detected. Interestingly, similar changes were seen at 37 mg/kg/day 4-MBC when administered by the dermal route. This result implies that the dermal route was 8.3 times more potent (or 8.3 times greater absorption via the dermal route) than the oral route for the induction of an uterotrophic effect. Included in the estimation of dermal absorption was an assumed penetration of 4-MBC of 0.4 to 0.6%, which could imply that less than 0.05 to 0.07% was absorbed orally. Studies with another UV-filter (benzophenone-3) have shown significant (AUC 98.7 µg/mL.h; Cmax 25.6 µg/mL) absorption and biliary circulation following oral administration. Another explanation for the difference in dose-response for the oral and dermal routes could relate to significant first-pass metabolism following oral administration that could be by-passed when the UV filter was absorbed via the dermal route. A lack of kinetic data with this study does not allow investigation of this possibility.

If significant first-pass metabolism of UV filters does occur then the oestrogenic impact of exposure through the food chain would be less likely to occur (if metabolites inactive). Interestingly, benzophenone-3 (UV filter studied following oral administration to rats) was strongly bound to plasma protein and significantly metabolised, with urine samples containing at least three metabolites.

The age (maturation status) and strain of the animals used was not justified; the strain used was different to the other assay carried out in rats and the age of animals used did not take into account the need to assess a spectrum of developmental stages.

## Possible kinetic issues

The detection of small amounts of sunscreen (with possible oestrogenic activity) in fish does not in itself establish a direct link to humans being exposed to increased levels of oestrogen-like agents. The sunscreen molecule with oestrogenic activity would be expected to undergo some degree of breakdown/metabolism from the time it is applied to the skin of a human to the time it is detected in a fish, and beyond in the preparation (cooking) of the fish for eating. An important question would be does the sunscreen molecule retain oestrogenic activity during the course of its deposition into the tissue of fish. The structure-activity relationship between oestrogen receptor activation and sunscreen molecule integrity may be altered following breakdown/metabolism. The assay used to detect the sunscreens in the tissue of fish could not be assessed for specificity, since this information was presented in a cited reference that was written in German.

Persistence of oestrogen-like agents in the body could be an issue. Oestrogen has a short life and is removed quickly from system, but if sunscreen agents resist breakdown they could pose a problem.

### Relationship to the Australian environmental situation

It was stated that in 1991 and 1993, six different UV screens were identified in fish of the Meerfelder Maar lake (Eifel, Germany) at total concentrations of up to 2 mg/kg (2 ppm) lipid in perch and 0.5 mg/kg (0.5 ppm) lipid in roach. Both fish species were contaminated with sunscreens, PCB,s and DDT at comparable levels. It was suggested that from these results it appears that UV screens are relevant environmental contaminants.

Points worth noting are that these findings (levels in fish) were for a lake (suggests enclosed body of water), where the movement of water and contaminants could be limited; substances can concentrate following evaporation and settling. This is unlike the great majority of situations where sunscreens are used in Australia, such as the beach with tidal flow resulting in a continuous exchange of water. Furthermore, it is likely that the environment/climate around this lake is colder (climate of Germany), which could result in slower breakdown of contaminants. Also, local fish species may maintain a high fat content (sunscreen stored in lipid) in the colder climate. It is likely that it is a fresh water lake, which would be a different reaction environment to the salt-water beaches in Australia.

# International reaction to date

In the **USA**, the EPA included a comment/summary of the study on their website, but have not indicated any further action. The initial information presented on the EPA website has now been moved to the archive section.

In **Europe**, COLIPA released a statement on UV-filters, which included a brief assessment of the findings of the study by Schlumpf et al (2001) described in this report. The statement indicated that, “on the basis of officially prescribed assessment methodologies and the available toxicological database, UV-filters are safe for topical application to humans in present practice of use and at officially prescribed maximum concentrations in cosmetics”.

The Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) assessed 4-MBC and concluded that it should be in classification 1. Classification 1 indicates that this substance for which data at the time of assessment support the conclusion that they do not pose a health hazard. The SCCNFP was of the opinion that, "the organic UV-filters used in cosmetic sunscreen products, allowed in the EU market today, have no oestrogenic effects that could potentially affect human health".

The SCCNFP expressed serious concerns as to the validity of the results published. The basis for these concerns is as follows:-

*In vitro* assay: The extremely low ($10^6$ lower) potency of the UV-filters compared with the positive control (oestradiol). The CSTEE (Scientific Committee on Toxicity, Ecotoxicity and Environment) consider *in vitro* assays as suitable only for screening and are not useful (based on excess of false negative/false positive results) for predicting *in vivo* endocrine disruptors. The *in vitro* ranking for the UV-filters going from Bp-3 to HMS did not correspond with the *in vivo* results.

*In vivo* assays: The OECD draft protocol on the rodent uterotrophic assay was not followed; moreover, GLP conditions were not applied. Deviations from the protocol included inappropriate rat strains used; exposure period of the rats ran to close to onset of puberty; the dermal exposure conditions were inappropriate; the calculation of absorbed dose following dermal exposure was unclear and the result excessive; the potency of the positive control (ethinylestradiol) was $10^5$ to $10^6$ greater than the UV filters, and the uterotrophic assay can only serve a limited function (is a short-term assay).

The Danish EPA advised that an assessment has been carried out of the results from the study by Schlumpf et al. There will be no change to the availability of sunscreens containing the 6 UV-filters (including 4-MBC) tested by Schlumpf et al for use by adults, but the agency has indicated that suntan lotions containing 4-MBC should not be used on children under 12 years of age.

# Conclusion

Overall, the issues raised in the Schlumpf article do not appear to require action at the present time.

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