Schlumpf et al. (1) reported on the in vitro and in vivo "estrogenicity" of six ultraviolet (UV) filters: benzophenone-3 (Bp-3), homosalate (H M S), 4-methyl-benzylidene camphor (4-M BC), octyl-methoxycinnamate (OM C), octyl-dimethyl PABA (OD-PABA), and butyl-methoxydibenzoylmethane (B-M DM). The authors concluded that "in vivo screens should be tested for endocrine activity, in view of possible long-term effects in humans and wildlife.

There is international consensus that in vitro data should serve only for screening purposes and that they are not suited for conclusions regarding risk assessment. The interpretation of the in vivo data presented is very much hampered by the fact that Schlumpf et al. (1) used nonstandard and non-GLP protocols, although official guidelines have been issued (2). Specifically, we refer to Schlumpf et al. 's choice of unusual rat strains (Long-Evans and N u rats) for the uterotrophic assay and to the mode of dermal administration (pups were totally immersed in oily solutions of the test compound). Because of the administration protocol used by Schlumpf et al. (1), the calculation of the absorbed dose after dermal exposure remains obscure. Also, the time of administration of the test compounds (postnatal day 26) was very close to or at the onset of puberty in most rat species.

Following established protocols and GLP procedures, a uterotrophic assay was performed in Sprague-Dawley rats (the standard strain) using three daily doses of 10, 100, or 1,000 mg/kg 4-M BC subcutaneously (3); no uterotrophic response was observed. In another uterotrophic assay (4), Bp-3 and OMC were tested in female immature Wistar rats. Bp-3 was administered in four oral doses of 500 and 1,000 mg/kg/day, and OMC was applied in three oral doses of 500 and 1,000 mg/kg/day; no uterotrophic effect was observed (4). Strain variations such as these are not entirely unusual.

According to Table 3 of Schlumpf et al. (1), effective oral doses (uterotrophic effect) were 0.342 µg/kg/day ethinylestradiol, 119 mg/kg/day 4-M BC, 1,035 mg/kg/day OMC, and 1,525 mg/kg/day Bp-3. The lower doses tested, (i.e., 0.085 µg/kg/day ethinylestradiol, 66 mg/kg/day 4-M BC, 522 mg/kg/day OMC, and 937 mg/kg/day Bp-3) must be regarded as no-hormonal-effect levels (NH ELS), based on the data of Schlumpf et al. (1). The effect of Bp-3 (called "weak" by the authors) appears in a range above the "limit dose," according to current Organisation for Economic Co-operation and Development guidelines (2).

A very weak effect of Bp-3 is not considered contradictory with negative findings in other studies (3), and it appears consistent with an estrogenic effect of the minor Bp-3 metabolite p-hydroxy-benzophenone (6), which comprises 1% of a benzophenone dose in rats (7).

Although the data of Schlumpf et al. (1) are in contrast to findings of others and have technical shortcomings, they can nevertheless be incorporated into risk assessment scenarios leading to worst-case views. We used the data presented by Schlumpf et al. in their Table 3 (1) as the basis of two assessments: a) we calculated a traditional margin of safety (MOS) based on the NH ELS observations of Schlumpf et al. and, b) we compared the estrogenic load that might be imposed on the human organism by the UV filters compounds under consideration with the estrogenic load imposed by phytoestrogens in the normal diet (hygiene-based margin of safety (H BM OS) (8).

Official exposure scenarios for 4-M BC and OMC have been described by the Scientific Committee of Cosmetic Products and Non-Food Products (SCCNFP) of the European Union as a basis of associated risk assessments (9,10).

The effects of Bp-3 in the study by Schlumpf et al. (1) are very much borderline if one considers that they are observed only at doses above the limit dose. Hence, the subsequent assessments are restricted to the two compounds (4-M BC and OMC) for which uterotrophic effects at lower doses have been reported by Schlumpf et al. (1).

With regard to human toxicity, experimentally based no-observed-adverse-effect levels (NO AEL) are the toxicologic key element. In contrast to other approaches, the MOS methodology of the European Union does not make use of numerically fixed assessment factors; the MOS is calculated by comparing the level of human exposure (estimated to a large extent by modeling) with the NOAEL from animal experiments.

Application of this concept to hormonally active compounds (endocrine modulators) is easily possible if the hormonal effect is considered the critical toxicity; this would mean that NH ELS could serve as specific substitutes of the NOAEL (11). In principle, this avenue of thinking has been advanced from the scientific side in discussions concerning regulations of hormonally active growth promoters in meat (12).

An MOS can be derived by comparing the NH ELS data of the two substances 4-M BC and OMC from Table 3 of Schlumpf et al. (1) with official exposure scenarios (systemic exposure doses) of the SCCNFP (Table 1) (9,10).

Bolt et al. (8) developed a supplementary route of comparative risk calculation using the concept of H BMOS. Basically, they compared exposure scenarios for individual industrial compounds with those of endocrine modulators of natural origin, under consideration of the respective relative potency ratios in vivo.

The dietary intake figures of estrogenic isoflavones have been assessed in our laboratory (8); data in the published literature are in general support of the scenario of the Senate Commission on the Evaluation of Food Safety of the Deutsche Forschungsgemeinschaft (SKL M) (13), which arrived at a human daily intake of isoflavones in the order of 1 mg/kg body weight.

Relative estrogenic potency assessment figures based on in vivo studies of 4-M BC and OMC, in relation to isoflavones (e.g., daidzein), can be derived from a synopsis of the results of the uterotrophic assays by Schlumpf et al. (1) (shown in their Table 3; the potencies of 4-M BC and OMC compared to that of ethinylestradiol) and by Bolt et al. (8). The latter data refer to uterotrophic assays by Diel et al. (14) that compare the potencies of the phytoestrogen daidzein and the reference compound ethinylestradiol, as well as other compounds.

Based on the concept of "dose additivity" for combinations of similarly acting compounds (15), Schlumpf et al. (1) provide data in their Table 3 of equally effective doses of ethinylestradiol, 4-M BC, and
Table 2. Calculations of MOSs by three different methods.

| Compound | 1. MOS [NHEL] | 2. MOS [NOAEL] | 3. HBMOS |
|----------|---------------|----------------|----------|
| 4-MBC    | 290           | 110            | 87       |
| OMC      | 870           | 750            | 167      |

Hermann M. Bolt
Christine Gruhe
Gisela H. Degen
Institute of Occupational Physiology at the University of Dortmund (IfADo)
Dortmund, Germany
E-mail: bolt@ifado.de

References and Notes

1. Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtenstein W. In vitro and in vivo estrogenicity of UV screens. Environ Health Perspect 109:239–244 (2001).
2. Insue T. Protocol for the Conduct of the OECD Rodent Uterotrophic Assay. Draft Protocol A. Tokyo: National Institute of Health Sciences, 2000.
3. Cometto L, Bussi R. Unpublished data.
4. Bachmann S, Hellwig J. Unpublished data.
5. Baker VA, Hepburn PA, Kennedy SJ, Jos on PA, Lee LJ, Sumpter JP, Ashby J. Safety evaluation of phytosterols esters. Assessment of oestrogenicity using a combination of in vivo and in vitro assays. Food Chem Toxicol 37:13–22 (1999).
6. Nakagawa T, Taya ma S. Estrogenic potency of benzo phenone and its metabolites in juvenile female rats. Arch Toxicol 75:74–79 (2001).
7. Stocklinski AW, Ware OB, Obstrell TH. Benzophenone metabolism. I. Isolation of p-hydroxyphenone from rat urine. Life Sci 26:385–396 (1980).
8. Bolt HM, Jan ning P, Michna H, Degen GH. Comparative assessment of endocrine modulators with oestrogenic activity. I. Definition of a hygiene-based margin of safety (HBMOS) for xenogenic agents against the background of European developments. Arch Toxicol 74:649–662 (2001).
9. SCC. Opinion of The Scientific Committee on Cosmetic Concerning 2-Ethylhexyl-4-methoxycinnamate (S28). Brussels: Scientific Committee on Cosmetic and Non-Food Products, 1998.
10. SCCNFP. Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concerning 3-(4-Methoxybenzylidene)-D-Limonene. Adopted by the Plenary Session of the SCCNFP of 1 January 1998. Brussels: Scientific Committee on Cosmetic Products and Non-Food Products, 1998.
11. Hoffmann B. Problems of testing and health risks of anabolic agents with sex hormone-like activities. In: Proceedings of the European Commission: Scientific Conference on Growth Promotion in Meat Production, Brussels, Belgium, 29 November–1 December 1995. Luxembourg: Directorate-General VI Agriculture, Office for Official Publications of the European Communities, 1996:271–296.
12. Lanning GE. Scientific working group reports on anabolic steroids in animal production. In: Proceedings of the European Commission: Scientific Conference on Growth Promotion in Meat Production, Brussels, Belgium, 29 November–1 December 1995. Luxembourg: Directorate-General VI Agriculture, Office for Official Publications of the European Communities, 1996:433–434.
13. Senate Commission of Food Safety of the Deutsche Forschungsgemeinschaft. Hormonal Active Agents in Food (Eisenbrand G, Daniel H, Dayan AD, Elias PS, Grunow W, Kemper FH, Lüsser E, Meßler M, Schläf R, eds). Weinheim, Germany: Wiley-VCH, 1998.
14. Dieri P, Schütz T, Smolnikar K, Strunck E, Vollmer G, Michna H. Ability of xenos- and phytotestogens to modulate expression of estrogen-sensitive genes in rat uterus: estrogenicity profiles and uterotrophic activity. J. Steroid Biochem Mol Biol 73:1–10 (2000).
15. Bolt HM, Muntau MM. Risk assessment of mixtures and standard setting: Working towards practical compromises. Food Chem Toxicol 34:1179–1181 (1996).

"In Vitro and in Vivo Estrogenicity of UV Screens": Response

We thank EHP for the opportunity to respond to the comments of Bolt and co-workers. Because Bolt et al. question our data on methodologic grounds and their use for risk assessment, we will deal with these two aspects separately.

Bolt et al. state that in vitro data should serve only for screening purposes and that they are not suited for conclusions regarding risk assessment.

This should be clear from our paper. In vitro experiments certainly are not meant to serve for risk assessment; however, it is generally accepted that positive results in vitro should lead to additional in vivo studies.

The oral experiments were conducted between spring and fall 1999. In contrast to Bolt et al.’s statement, there is still not an official guideline for the uterotrophic assay. The Endocrine Disrupters Testing and Assessment (EDTA) Task Force of the Organisation for Economic Co-operation and Development (OECD) decided on a proposal for the preparation of a guideline at its meeting held 26–27 May 2001.

Bolt et al. believe that our use of Long-Evans rats was not acceptable for our study. To our knowledge, the OECD protocol (1) will not specify the rat strains to be used for the uterotrophic assay. Long-Evans rats have long been used in neuroendocrine and endocrine investigations. The hairless (hr/hr) strain was recommended to us for dermatologic studies by a European breeding institute. Because we used this strain, we were able to use Aghazarian et al.’s (2) recent rat skin penetration data, which were obtained on skin of the same strain, for a provisional estimation of dermal dosage.

The hr/hr rats are derived from the O.F.A (Oncins France)-SD (Sprague Dawley) strain (IFSA CREDO, L’Arbresle, France).

We used dermal application by immersion in warm olive oil after discussing the method with other endocrine disruptor experts. We chose this method mainly for the following reason: The surface on the back of the immature animal available for application of compound is much smaller than in adult rats, and plasters that would not unduly disturb the immature animals could easily be removed by the littermates in the cage. When working with immature animals, a major requirement is avoidance of stress. The OECD protocol (1) also recommends group housing “because single housing of immature animals may cause considerable stress.”
immersion, carried out very gently, was well tolerated by the animals.

Irrespective of the method used to calculate the dermally applied dose, the amount taken up by the animal can never be calculated with the same precision as with the oral or parenteral routes from the amount applied. The only solution is the determination of blood and tissue levels, which presently is in progress in our laboratory using gas chromatography-mass spectrometry.

As we stated in our paper (3), experiments were conducted on offspring of time-pregnant rats. We recorded birth (occurring in the morning of gestational day (GD) 23, which is also postnatal day (PN) 1: GD 1 = 24 hr after mating period); pups were then culled to eight per litter and observed daily. The development of uterine weight was studied in detail between PN 20 and PN 32 in Long-Evans and hairless rats (4). These data clearly show that in both strains, uterine weight remains at the same level until PN 26. Thus, PNs 25 and 26 can be used in our strains. This is also evident from an analysis of histograms of frequency distributions of individual uterine weights in controls and treated groups from oral and dermal studies investigated at PN 25 and 26 (5).

With respect to OMC and Bp-3, our data are in full agreement with the data quoted by Bolt et al. The oral no-hormonal-effect levels Bolt et al. compared to our data were as follows: 250 vs. 522 mg/kg/day for OMC and 1,000 vs. 937 mg/kg/day for Bp-3. The lowest-observable-effect levels were 103 mg/kg/day for OMC and 1,525 mg/kg/day for Bp-3 in our oral study. The only difference is with 4-MBC, where no effect was seen after treatment with up to 1,000 mg/kg/day in the study quoted by Bolt et al., whereas we observed a significant increase in uterine weight at 119 mg/kg/day. Apart from rat strain, the main difference between the two studies is the route of application, subcutaneous versus oral (2). Even though the subcutaneous route is thought to be slightly more sensitive in the uterotrophic assay, this may not be the case for all compounds. Controversial uterine weight data also exist for other chemicals with proven binding and transcriptional activity at estrogen receptors. This issue cannot yet be considered to be completely settled. In some cases with negative uterine weight data, other estrogen-sensitive parameters were influenced by the compound (6). Recently, we corroborated increased uterine cell proliferation following dermal application of 4-MBC and OMC by demonstrating increased bromodeoxyuridine uptake (4,5).

In our view, Bolt et al. make inappropriate use of our data. There is international agreement that the uterotrophic assay can only serve a limited function as a test for in vivo identification of chemicals with estrogenic (or antiestrogenic) activity. To our knowledge, the uterotrophic assay is situated between in vitro screening tests and long-term studies (e.g., TG 416). In the scenario for the investigation of endocrine disruptors conceived by the EDTA committee of the OECD, which initiated the validation of this test. The uterotrophic assay typically is an acute high-dose test. As discussed in detail in our paper (3), other known xenobiotics such as mechoxynol, nonylphenol, bisphenol A, and o,p'-DDT also need to be applied at similar high doses to achieve a significant growth of the uterus in a few days (7–10). If one considers the complex organizational and activation actions of steroid hormones at different stages of the life cycle, it becomes clear that such acute data cannot provide a basis for long-term risk calculations. In view of possible differences in gene regulation patterns, it is also not possible to draw conclusions on long-term risk from a comparison of dosages of different estrogenic chemicals.

At first sight, it seems tempting to relate estrogenic activities of different chemicals to a phytoestrogen occurring in food and to use this for a unified evaluation of “in vivo estrogenicity.” However, this concept presents serious scientific flaws, in particular with regard to an application to long-term effects. It is evident from the scientific literature of the last 10–15 years that estrogenic chemicals are not alike. First of all, there are important differences in toxicokinetics that are not considered by comparing daily intake. In addition, and this may be more important, estrogenic chemicals can differ markedly in their effects on gene regulation, not only quantitatively but also qualitatively. It is well known that even closely related chemicals, such as tamoxifen and roloxifen, exhibit different tissue selectivity, acting as agonist (tamoxifen) or antagonist (roloxifen) in one and the same tissue (uterus) (11). Moreover, different compounds with agonistic or partial agonistic activity at estrogen receptors can recruit different coactivators/corepressors (11,12), and elicit different gene induction patterns, for example, in the uterus (13). In a recent study on adult ovariectomized rats (14), the effect of diadzein on mRNA expression in uterus differed qualitatively (up- or down-regulation) from that of bisphenol A for three out of six genes studied, and for DDT for two out of six genes. These differences in effects on gene regulation are not consistently revealed by simple measures of estrogenicity such as proliferation of cell lines or uterine growth. They may well matter in particular with long-term exposure. As a consequence, the principle of using a phytoestrogen such as daidzein (or ethinylestradiol) as a reference compound for estrogenic activity and extrapolating from the activity ratio calculated from acute data to long-term risk of chemicals with a different structure cannot be considered to represent a valid approach.

In our paper (3), we reported on acute in vitro and in vivo estrogen-like effects of UV screens. We deliberately abstained from extrapolation of these acute data to long-term exposure in view of risk assessment because this could not be considered to be scientifically sound on the basis of present knowledge. Much to our surprise, this is being done now by colleagues from both academia and industry. In our view, the calculation of safety margins for chronic exposure from our acute data is not acceptable on scientific grounds.

We insist that the effect levels of five UV screens in vitro and three compounds in the uterotrophic assay are in the range of other chemicals for which there is general agreement on the need for long-term studies on possible endocrine effects. Hence, we think that a solid risk assessment requires additional long-term studies, with particular reference to endocrine parameters, and a more detailed analysis of acute and chronic toxicokinetics.

Margret Schlumpf
Walter Lichtensteiger
Institute of Pharmacology and Toxicology
University of Zurich
Zurich, Switzerland
E-mail: schlumpm@pharma.unizh.ch

REFERENCES AND NOTES

1. Protocol for the Conduct of the OECD Rodent Uterotrophic Assay. Unpublished data.

2. Aghazarian V, Tchikake L, Reynier JP, Gayte-Sorbier A. Release of benzimidazole and benzyldiene camphor from topical sunscreen formulations. Drug Dev Ind Pharmacy 25:1277–1282 (1999).

3. Schlumpf M, Cotton B, Conscience M, Hailer V, Steinmann B, Lichtensteiger W. In vitro and in vivo estrogenicity of UV screens. Environ Health Perspect 109:239–244 (2001).

4. Schlumpf M, Berger L, Cotton B, Conscience-Egli M, Durrer S, Fleischmann I, Hailer V, M Arik K, Lichtensteiger W. Estrogen active UV screens. Seifen-Öle-Fette-Wachse (in press).

5. Berger L. Prepubertal Development of the Female Rat: Uterotrophic Response to Dermal Application of the UV Screens MBC, OMC, BP-3 and HMS [Diploma Thesis]. Zürich Universität of Zurich, Faculty of Mathematical and Natural Sciences, 2001.

6. Gould J, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonell DP, Caído KW. Bisphenol A interacts with the estrogen receptor α in a distinct manner from estradiol. Mol Cell Endocrinol 142:203–214 (1998).

7. Metcalf J, Laws SC, Cummings AM. Methoxychlor mimics the action of 17β-estradiol on induction of uterine epidermal growth factor receptors in immature female rats. Reprod Toxicol 10:395–399 (1996).

8. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL.
Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environ Health Perspect 104:1296–1300 (1996).
9. Odum J, Jelleve PA, Tittersson S, Patten D, Routledge EJ, Beresford NA, Sumpter JP, Ashby J. The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenicity assay. Regul Toxicol Pharmacol 25:176–188 (1997).
10. Ashby J, Tinwell H. Uterotrophic activity of bisphenol A in the immature rat. Environ Health Perspect 106:719–720 (1998).
11. Dutertre M, Smith CL. Molecular mechanisms of selective estrogen receptor modulator (SERM) action. J Pharmacol Exp Ther. 295:431–437 (2000).
12. Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB. The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11:693–705 (1997).
13. Nephew KP, Polek TC, Akcali KC, Khan SA. The antiestrogen tamoxifen induces c-fos and jun-B, but not c-jun or jun-D, protooncogenes in the rat uterus. Endocrinology. 133:419–422 (1993).
14. Diel P, Schulz T, Smolnikar K, Strunck E, Vollmer G, Michna H. Ability of xeno- and phytoestrogens to modulate expression of estrogen-sensitive genes in rat uterus: estrogenic profiles and uterotrophic activity. J Steroid Biochem Mol Biol 73:10 (2000).

The Full Circle: From the Minamata Disaster to the Sick Building Syndrome

Pekkanen and Pearce (1) recently focused on the challenges and opportunities of environmental epidemiology. Their paper recalls to our minds the fascinating story of humans and environment: they continuously look for the best milieu for their lives. First, they think that industrialized areas are better than natural areas; then they reason that returning to the natural environment is probably better, always being sure that each choice is the safest. However, no choice allows environmental risks to be completely abolished, and the only way to cope with the problem of environmental risks is to face them [as Pekkanen and Pearce (1) did, by looking for the best studies to evaluate these risks], not to believe they have been blunted.

Can you imagine a paradise better than that of Minamata Bay, facing the Shiranui Sea in Japan? It has blue sea, white sand, green shrubs, burning sun, and bright stars. But in the mid-1950s, some unexplainable occurrences brought panic to Minamata: birds were strangely dropping from the sky, cats committed “suicide,” and people began to notice a “strange disease” that caused numbness in limbs and lips, slurring of speech, vision constriction, uncontrollable shouting, involuntary movements, and unconsciousness.

The risk came from 27 tons of mercury compounds dumped into Minamata Bay from 1932 to 1968 by a company developing plastic, drugs, and perfumes, through the use of acetaldehyde, which is produced using mercury. Over 3,000 victims suffering from degeneration of the nervous system have been recognized as having Minamata disease (2,3).

Paradise was only a dream; good health in a pure, uncontaminated area cannot continue in the absence of safety controls.

Humans thought they had learned the lesson and began to construct safer buildings, as a modern paradise with many comforts and far from environmental risks. But, in the mid-1970s, some unexplainable occurrences brought concern: people living in recently built houses began to suffer somatic and psychological symptoms, including arthralgia, eye and throat irritation, cough, rash, pruritus, enhanced and/or abnormal odor perception, visual disturbances, mild to severe headache, nausea, vomiting, restlessness, and sleeplessness. Some volatile component of the building materials or some biological contaminant (perhaps endotoxin, mycotoxin, or trace elements) might be causing this unique systemic syndrome, the so-called sick building syndrome (4,5).

This constructed perfection was also a dream. Good health in an artificial, sophisticated structure is not guaranteed even in the presence of better safety controls, or perhaps by the presence of modern technological devices such as humidifiers and ventilation systems.

Nature is less perfect and more vulnerable than we used to surmise; for humans living on the earth crust, each new direction has its disadvantages. Can people win against the environment? Looking at environmental epidemiology with its opportunities and challenges (1) is a largely better approach than that of dreaming about unlikely simple and perfect solutions, such as that of coming back to pure, uncontaminated nature or waiting for a completely technology-modified environment.

A human life, either “natural” or industrialized, has some challenges, as both the Minamata disaster and the sick building syndrome demonstrate. A concern for sick building syndrome does not justify the claim that pure uncontaminated nature (including Minamata Bay or fresh unsterilized milk often containing tuberculosis bacteria) is the best goal for humans. A logical and scientific approach to the problem, such as that offered by Pekkanen and Pearce (1), must be shared because it offers the only possibility for humans to survive. Living without risks is impossible, but lowering the threshold of risks is necessary.

Piero Stratta
Alessandra M esserotti
Caterina Canavesi
Department of Internal Medicine
S. Giovanni M oniello Hospital
Torino, Italy
E-mail: strattanefro@hotmail.com

REFERENCES AND NOTES

1. Pekkanen J, Pearce N. Environmental epidemiology: challenges and opportunities. Environ Health Perspect 109:1–5 (2001).
2. TED Case Studies. Minamata Disaster. Available: http://gurukulu.ucc.american.edu/TED/minamata.htm [cited 1 August 2001].
3. Minamata, and then... Available: http://gurukulu.ucc.american.edu/TED/minamata.htm [cited 1 August 2001].
4. Bourbeau J, Brisson J, Allaire S. Prevalence of the sick building syndrome symptoms in office workers before and after being exposed to a building with an improved ventilation system. Occup Environ Med 53:204–210 (1996).
5. Redlich CA, Sparer J, Cullen MR. Sick building syndrome. Lancet 349:1013–1016 (1997).