

Shining a Light on Sunscreens

From a historical perspective, endocrinology used to be one of the most integrative disciplines in organismal biology. However, the extraordinary advancement attained during the last 30 yr about the role of steroid receptors as transcription factors brought to endocrinology a reductionistic attitude. This resulted in the conviction that if the basic mechanisms of transcription regulation were understood, we would be able to achieve a full understanding of hormone action. Yet, this view did not take into consideration the fact that biology is a historical science. Moreover, as Francois Jacob pondered, evolution operates as a tinker, not as an engineer. Mechanisms are not invented *de novo*, but rather old elements are put to new use during evolution. From this perspective, data that do not fit the dominant paradigm should be of utmost concern because they may be exposing a gap in our knowledge that could provide important clues to the very phenomena that we thought we understood. Endocrine disruptors provide this challenge and opportunity.

The term “endocrine disruptors” was coined at a conference entitled “Chemically Induced Alterations in Sexual Development: The Wildlife/Human Connection” held in 1991 at the Wingspread Conference Center in Racine, WI. The conferees’ objective was to address an emerging issue in wildlife biology: adult animals appeared to be healthy, whereas their offspring were plagued with a panoply of effects that suggested abnormal development, a phenomenon that was well documented in the Great Lakes environment and supported by studies in other ecosystems (1). Many of these effects were linked to alterations of the endocrine system, and, hence, the chemicals involved were called endocrine disruptors (2).

The estrogenic activity of nonylphenol and bisphenol-A was revealed because they migrate from plastic labware, thus disrupting laboratory experiments (3, 4). Because most of these compounds bind to estrogen receptors with lower affinity than the natural ligands, the mainstream thinking was that they did not pose problems for human health, this despite widespread exposure to these agents (5). Thus, it was thought that wildlife was affected merely because of the dumping of large quantities of these weak hormone-like chemicals into some watercourses. However, the rise in breast cancer incidence and malformations of the male genital tract during the last 50 yr (6, 7), coincidental with the massive introduction of endocrine disruptors into the environment, inspired Wingspread participants as well as other scientists to ignore the commonsensical, but mistaken, assurance that weak hormones at low doses were harmless.

Abbreviations: BPA, Bisphenol-A; ER, estrogen receptor; 4-MBC, 4-methylbenzylidene camphor; PR, progesterone receptor.

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Instead, they posited that fetal exposure to environmentally relevant doses of endocrine disruptors may produce effects that, like those of prenatal exposure to diethylstilbestrol, would be manifested long after exposure ended. Indeed, fetal exposure to xenoestrogen bisphenol-A (BPA) at doses several orders of magnitude lower than that needed to increase the weight of the uterus in ovariectomized adults was found to alter the morphology of several hormone-target organs such as the uterus, vagina (8), mammary glands (9), and the prostate (10). Perinatal exposure to BPA also advanced puberty in females (11), disrupted estrus cyclicity (12, 13), and elicited aging of the hypophyseal-pituitary-ovarian axis (13). Furthermore, developmental exposure to BPA also affected the behavior and induced obesity in exposed animals (13, 14).

These recent findings spurred, the exploration of phenomena occurring at the lower levels of biological organization, such as receptor binding and gene activation, to identify the probable mechanisms that would explain why fetal exposure to xenoestrogens produce effects at doses significantly lower than those predicted from their estrogen receptor (ER) binding affinity. A potential explanation was provided by the finding that xenoestrogens act additively with endogenous hormones (15). Another potential explanation for low-dose effects arose from studies on cell membrane steroid receptors. This study found that a series of estrogens that had a low relative binding affinity to nuclear ER α and β were as potent activators of membrane-bound receptors (16). On the other hand, interest in phenomena occurring at higher levels of biological organization resulted in renewed awareness by developmental biologists of the role of estrogens during organogenesis (17), a subject once believed to be irrelevant after it was found that the ER α null mutant seemed to have undergone apparently “normal” fetal development of the genital tract and mammary gland (18).

Now, in this issue, Durrer *et al.* (19) report on the effects of developmental exposure to the estrogen agonist 4-methylbenzylidene camphor (4-MBC), a chemical widely used in sunscreens. These authors had recently identified as estrogen agonists a series of chemicals included in cosmetics and other applications (20). Although there are no quantitative data on the levels of these chemicals in human tissues, humans are exposed continually to these chemicals through personal care products, washing powder, carpets, and many more products present in households. Because these compounds are lipophilic, they are likely to bioaccumulate. Interestingly, the 4-MBC doses used in this study are close to the levels found in roach and perch in Swiss lakes. The presence of these compounds in wildlife is of relevance not only from the ecological viewpoint, but also because it may indirectly increase human exposure through the food chain.

Durrer *et al.* (19) part ways with the approach used to assess the developmental effects of BPA in that they exposed the parents before mating and the F1 generation from conception to adulthood, thus mimicking the ways we humans

are exposed. Exposure of males to 4-MBC resulted in decreased testis weight at postnatal d 14, delayed puberty, and decreased adult prostate weight. In females, it increased adult ovarian and uterine weight. The weight of the thyroid was increased in both female and male adult animals. The aim of this work was not to find out how this exposure led to the alterations found in the exposed animals, but rather to assess whether during chronic exposure to 4-MBC there were changes in the expression of estrogen-regulated genes in the uterus of females examined at diestrus. 4-MBC altered the steady-state levels of mRNAs encoding for ER α , progesterone receptor (PR), IGF-I, and androgen receptor, thus suggesting potential alterations of the response to those hormones as well. These effects may result from either the agonistic activity of 4-MBC present at the time of the experiment, an indirect effect taking place during development, or a combination of both. The animals exposed to 4-MBC showed a significant reduction in the response to estradiol after ovariectomy for all the gene products studied. This decreased sensitivity may be explained by the significant decrease in protein level of the steroid receptor coactivator-1; this is an interesting finding, because this coactivator does not seem to be regulated by acute administration of estrogen agonists. However, the opposite effect, namely increased sensitivity, was found when the effect of estradiol was assessed in the ventromedial hypothalamus of the same animals.

What have we learned from this study? First, in general, most of the disrupting effects at lower levels of biological organization are manifested at the same doses that produce a significant effect at a higher level of organization. That is, significant changes in mRNA levels and weight of the uterus both occur at 24 mg/kg. Yet, in the particular cases of the PR-A and the steroid receptor coactivator-1 proteins significant changes were observed at lower doses than those required for increased uterine wet weight. Second, the relationship between dose and effect is not always monotonic (*i.e.* lower doses produce larger effects than higher doses), a phenomenon frequently associated with perinatal exposure to xenoestrogens. Third, the relationship between the level of a given mRNA and its product is not straightforward; that is, the protein level was modified at a lower dose of 4-MBC than was the mRNA level. Finally, the changes at the gene expression level do not suggest an explanation for the changes at the high level; how do lower levels of ER α , PR, and IGF-I, and decreased sensitivity to estradiol explain an increased uterine weight?

With hindsight, it is understandable that the complexity of the organism results in an apparent discontinuity between low- and high-level phenomena. This problem has occupied historians and philosophers of biology for a long time (21). While leaving to philosophers to argue whether this represents a real discontinuity or an apparent one that will be bridged by new knowledge, we bench scientists have to tackle this problem now (22). Rather than ignoring this problem by concentrating only on the low level phenomena, it will be equally productive to pay attention to the higher level of biological organization as well. Finally, in fact, we cannot avoid studying the higher level phenomena, for it is imperative to know which are the health risks resulting from ex-

posure to endocrine disruptors. This is particularly relevant in the case of sunscreens. Contrary to the vast majority of xenoestrogens, exposure to sunscreens is a personal choice fostered by the public health community that wants to prevent skin cancer, a controversial subject in its own right (23). The paper from Durrer *et al.* (19) illustrates the unintended consequences of this exposure, *i.e.* increased uterine weight, altered estrogen sensitivity, and altered gene expression in estrogen target organs. Other alterations, such as decreased thymus weight and increased thyroid weight, are also likely due to the estrogenic properties of 4-MBC (24). These findings pose troubling questions to those responsible for setting public health policy. Of obvious, immediate importance is to assess whether the observed effects of 4-MBC exposure result in impaired reproduction, immune, and thyroid function. Another consideration, of evolutionary importance, is whether continuous exposure to these chemicals will represent a selective disadvantage for exposed progeny.

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References

1. Colborn T, Clement C, eds. 1992 Chemically induced alterations in sexual and functional development: the wildlife/human connection. Princeton, NJ: Princeton Scientific Publishing
2. Colborn T, vom Saal FS, Soto AM 1993 Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101: 378–384
3. Soto AM, Justicia H, Wray JW, Sonnenschein C 1991 p-Nonyl-phenol: an estrogenic xenobiotic released from “modified” polystyrene. *Environ Health Perspect* 92:167–173
4. Krishnan AV, Starhis P, Permuth SF, Tokes L, Feldman D 1993 Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* 132:2279–2286
5. Safe S 1995 Environmental and dietary estrogens and human health: is there a problem? *Environ Health Perspect* 103:346–351
6. Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H 1993 Medical hypothesis: xenoestrogens as preventable causes of breast cancer. *Environ Health Perspect* 101:372–377
7. Sharpe RM, Skakkebaek NE 1993 Are oestrogens involved in falling sperm count and disorders of the male reproductive tract? *Lancet* 341:1392–1395
8. Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM 2 February 2005 Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. *Biol Reprod* 10.1095/biolreprod.104.0360301
9. Markey CM, Luque EH, Munoz de Toro MM, Sonnenschein C, Soto AM 2001 In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 65:1215–1223
10. Welshons WV, Nagel SC, Thayer KA, Judy BM, vom Saal FS 1999 Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. *Toxicol Ind Health* 15:12–25
11. Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenberg JG, vom Saal FS 1999 Exposure to bisphenol A advances puberty. *Nature* 401:763–764
12. Markey CM, Coombs MA, Sonnenschein C, Soto AM 2003 Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. *Evol Dev* 5:1–9
13. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM 2001 Perinatal exposure to low doses of bisphenol-A affects body weight, patterns of estrous cyclicity and plasma LH levels. *Environ Health Perspect* 109:675–680
14. Farabolini F, Porrini S, Della Seta D, Bianchi F, Dessì-Fulgheri F 2002 Effects

- of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. *Environmental Health Perspectives* 110(Suppl):409–414
15. **Silva E, Rajapakse N, Kortenkamp A** 2002 Something from “nothing”—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environ Science Technol* 36:1751–1756
 16. **Wozniak AL, Bulayeva NN, Watson CS** 25 January 2005 Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor- α mediated Ca^{++} fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ Health Perspect* 10.1289/ehp.7505
 17. **Kitajewski J, Sassoon DA** 2000 The emergence of molecular gynecology: homeobox and Wnt genes in the female reproductive tract. *Bioessays* 22:902–910
 18. **Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O** 1993 Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 90:11162–11166
 19. **Durrer S, Maerkel K, Schlumpf M, Lichtensteiger W** 2005 Estrogen target gene regulation and coactivator expression in rat uterus after developmental exposure to the ultraviolet filter 4-methylbenzylidene camphor. *Endocrinology* 146:2130–2139
 20. **Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger W** 2001 In vitro and in vivo estrogenicity of UV screens. *Environ Health Perspect* 109:239–244
 21. **Rosenberg A** 1994 *Instrumental biology, or, the disunity of science*. Chicago, IL: University of Chicago Press
 22. **Soto AM, Sonnenschein C** 27 September 2004 Emergentism as a default: cancer as a problem of tissue organization. *J Biosci* (Special Issue on Genetic Determinism)
 23. **Vainio H, Bianchini F** 2000 Cancer-preventive effects of sunscreens are uncertain. *Scand J Work Environ Health* 26:529–531
 24. **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** 2004 Endocrine activity and developmental toxicity of cosmetic UV filters—an update. *Toxicology* 205:113–122

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