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Reproductive stimulation by low doses of xenoestrogens contrasts with the view of hormesis as an adaptive response

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We discuss the similarities and differences of two types of effects that occur at low but not high doses of chemicals: hormesis and stimulation by oestrogenic endocrine-disrupting chemicals or xenoestrogens. While hormesis is a general phenomenon evoked by many compounds, oestrogenic stimulation occurs for specific chemicals that disrupt actions of endogenous oestrogen. Both types of phenomena can induce an inverted-U dose–response curve, resulting from low-dose stimulation of response, and thus challenge current methods of risk assessment. Hormesis is generally thought to be caused by an over-reaction of detoxification mechanisms, which is considered an adaptive response that should protect an organism from subsequent stress. One view of the hormetic low-dose stimulatory response, i.e., increased performance, is that it is beneficial. In contrast, we propose that for manmade xenoestrogens this is never the case. This is demonstrated with examples for low doses of the oestrogenic environmental chemicals bisphenol A and octylphenol, and the oestrogenic drug diethylstilbestrol. Adverse low-dose effects include oviduct rupture, an enlarged prostate, feminization of males and reduced sperm quality. These adverse stimulatory effects divert energy needed for other processes, resulting in reduced fitness. In conclusion, while there are similarities (inverted-U dose-response), there are also differences, adaptive response for hormesis versus adverse stimulatory response for low doses of manmade xenoestrogens, that have been almost totally ignored in discussions of hormesis. We propose that the risk posed by low doses of manmade xenoestrogens that show inverted-U dose–response curves is underestimated by the current threshold model used in risk assessment, and this is likely to apply to other endocrine-disrupting chemicals. *Human & Experimental Toxicology* (2005) **24,** *431–437*

Key words: bisphenol A; dynamic energy budget; endocrine disruption; hormesis; inverted-U; xenoestrogen

Introduction

A recent scientific debate concerns the occurrence and evaluation of hormetic responses in toxicology.[1–3] Hormesis is proposed to involve overcompensation that occurs after a toxic insult as homeostasis is re-established after being disrupted. The stimulatory event is presumably a result of an overallocation of resources relative to what is needed for repair processes and it is presumed to be adaptive in that it insures that the repair occurs and protects against the possibility of subsequent insult that might occur shortly after the first insult. The defining characteristic of hormesis is a stimulation of performance resulting from exposure to low concentrations of chemicals that are toxic at higher doses.[4] Therefore, hormesis might be incorrectly assumed to be based on the same mechanisms involved in the increase in reproductive effort or increase in organ or body size demonstrated by some organisms exposed to low doses of manmade oestrogenic endocrine-disrupting chemicals (manmade xenoestrogens).

In this paper our objective is to fuel the current discussion by addressing the distinct similarity (the presence of inverted-U dose–response relationships), as well as differences between responses to low doses of manmade xenoestrogens, which are uniformly unfavourable, and hormesis, where responses are typically viewed as adaptive. We will provide some experimental examples of oestrogenic responses to chemicals with oestrogenic activity, which unequivocally do not fit the assumption of an

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adaptive response used to describe hormesis.[1,2] Recognition and assessment of an oestrogenic response lies at the basis of manmade xenoestrogen risk assessment and is therefore of the utmost importance. In our opinion, it would be a critical mistake to apply the assumptions regarding hormesis to what we believe are clear examples of adverse effects caused by low doses of manmade xenoestrogens and other endocrine-disrupting chemicals. Implementing the hormesis concept in risk assessment would have an enormous impact, because it would imply that exposure to low doses of toxic chemicals could be good for the organism, a view that has led to the suggestion that risk assessment is overprotective and is causing unnecessary fear of exposure to low doses of chemicals.[2]

The response systems for oestrogen evolved to enable responses to endogenous oestrogen. These receptor systems do not have evolved mechanisms that automatically permit discrimination as to whether the stimulation is occurring due to endogenous or exogenous oestrogen. Thus, stimulation does not lead to initiation of repair processes. Oestrogens are mitogens and can stimulate cell proliferation at very low doses.[5] The view that hormesis is a tightly regulated slight overcompensation of repair processes, and is thus an adaptive mechanism,[1] has no relevance for oestrogenic stimulatory responses initiated by low doses of manmade xenoestrogens within a physiological range of oestrogenic activity.[5] This is particularly important in fetuses where homeostatic systems are being established and are not fully functional. We are unaware of any data showing that responses to low doses of manmade xenoestrogens in the environment are beneficial when all responses over the organism's life span are considered. Responses to low doses of manmade xenoestrogens include disruption of the functioning of cells, impaired organ function, disruption of homeostasis, exhaustion of an organism's energy budget and even an increased mortality rate.[6]

Hormesis versus response to low doses of manmade xenoestrogens

For a correct understanding of the debated issue, clear and simple definitions of the hormetic and oestrogenic response are required and are provided below.

A *hormetic response* is the stimulatory response shown by an organism exposed to low concentrations or doses of toxicants, while inhibition of response occurs at much higher doses. Stimulation at low doses is most likely caused by an overreaction of an organism's detoxification mechanism,[1] which stimulates its entire metabolism, leading to a performance exceeding that of organisms in the control group. Hormesis is attributable to an array of possible working mechanisms and has been found to occur for many endpoints in a wide variety of organisms. It may well have resulted from evolutionary adaptation of organisms to toxic substances present in their environment. Cases of hormesis have been documented for numerous different chemicals.

Oestrogenic responses to natural or manmade oestrogenic chemicals can be stimulated at low doses and inhibited at high doses, similar to the dose–response relationship described as hormesis.[5] In contrast to hormesis, oestrogenic responses are evoked by specific chemicals. These chemicals exert their effects by either mimicking oestradiol (direct effects) or by interfering with the production, metabolism and transport of oestradiol and interfering with oestrogen receptors (indirect effects). Manmade chemicals classified as xenoestrogens have to meet certain structural requirements to be able to bind to the oestrogen receptor or interfere with a specific component of oestrogen biology.[5] Thus, the *oestrogenic response* is a specific effect, such as stimulation of the female reproductive system, that occurs through interaction of a chemical with the classical nuclear oestrogen receptor (alpha and beta) or via more recently discovered receptors associated with the rapid induction of second messenger systems. Such interactions may lead to an increased number of eggs or offspring, which is a typical oestrogenic response observed in female molluscs exposed to low concentrations of manmade xenoestrogens, such as bisphenol A (BPA), 4-*tert*-octylphenol and 17α-ethinylestradiol. These chemicals all mimic the natural hormone oestradiol.[7–9] A typical response in female mammals is stimulation of the uterus and other reproductive tissues at low but not high doses.[10–12] The situation in males is more complicated, with some reproductive organs being stimulated (prostate) and others inhibited (testes, epididymides and seminal vesicles) in some species.[9,13,14]

Examples of effects of manmade xenoestrogens

To illustrate the type of effects and typical inverted U-shaped concentration–response curves that result from exposure to manmade xenoestrogens, three examples are provided here. These are presented because they might be confused as being an adaptive response due to being categorized as hormesis.

1. A 96-hour life-cycle test was conducted with the nematode *Caenorhabditis elegans* and 4-*n*-octylphenol (0.1–1000 nM). *C. elegans* was chosen for this assessment because it possesses an oestrogen receptor.[15] A significant increase in the number of juveniles per adult was observed for concentrations up to 100 nM (Figure 1a). At 1000 nM, the number of juveniles per adult had returned to the control level. Figure 1b shows the accompanying growth (body length) of the exposed nematodes, which was significantly inhibited at all tested concentrations. For the concentrations 0.1–100 nM, the reduced body length may have been the result of allocating the energy to reproduction, rather than to growth; observations that are supported by the Dynamic Energy Budget theory.[16] At 1000 nM, 4-*n*-octylphenol has probably reached a toxic level, as it no longer stimulates reproduction but still inhibits growth.

2. In females of the gonochoristic prosobranch snail species *Marisa cornuarietis*, BPA and 4-*tert*-octylphenol induce a complex syndrome of alterations referred to as 'superfemales', even at concentrations as low as 1 µg/L.[9] Affected specimens are characterized by the formation of additional female organs, an enlargement of the accessory pallial sex glands, and a massive stimulation of egg and clutch production (Figure 2a). This stimulation of egg production during the sexual repose phase of the snails is detrimental to the affected females, because it causes a congestion of clutches in the pallial oviduct, leading to a rupture of the oviduct and ultimately to the female's death. Up to 15.4% of all dissected females exposed to BPA or 4-*tert*-octylphenol exhibited these oviduct ruptures, but the incidence of these malformations was assumed to be much higher. This was deduced from the significant increase in mortality for all BPA and 4-*tert*-octylphenol treatments (Figure 2b), which is most likely caused by oviduct ruptures. The indication for a female-specific mortality in the exposure groups is supported by a slight, although not statistically significant, shift in the sex ratio of surviving animals in favour of males.[9] The reproductive stimulation by BPA in *M. cornuarietis* and the associated mortality are mediated by oestrogen receptors, as both effects are fully suppressed in the presence of the anti-oestrogens (competitive oestrogen receptor antagonists) tamoxifen and ICI 182,780.[17]

3. An inverted-U dose–response relationship for the oestrogenic drug diethylstilbestrol (DES) administered to pregnant mice (*Mus musculus domesticus*) on the development of prostate ducts during fetal life and subsequent prostate size and androgen receptor numbers has been shown.[18–20] At maternal oral doses of 0.02, 0.2 and 2.0 µg/kg body wt/day,

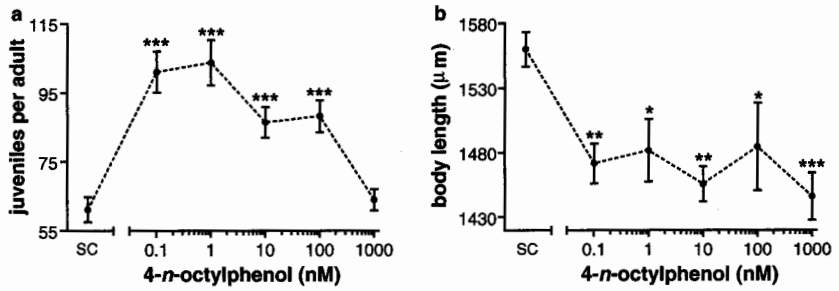

Figure 1 Effects of 4-*n*-octylphenol on nematode (*Caenorhabditis elegans*) reproduction (a, left) and growth (b, right). SC, solvent control. Symbols are means ($n=6$, for SC $n=11$) with standard error. Asterisks denote significant differences from the solvent control ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, according to Dunnett's post hoc test following one-way ANOVA).

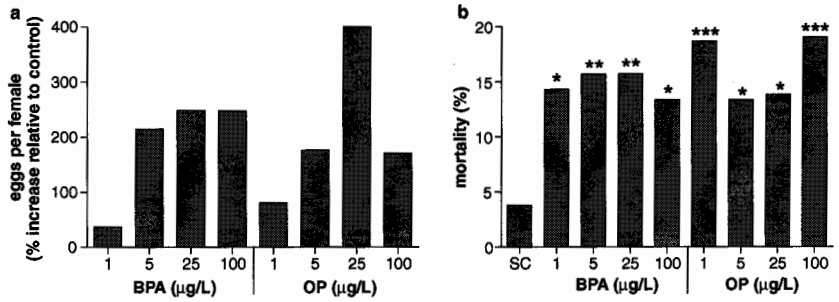

Figure 2 Effects of BPA and 4-*tert*-octylphenol (OP) on ramshorn snail (*Marisa cornuarietis*) reproduction (a, left) and mortality (b, right). Each exposure group consisted of 240 specimens. SC, solvent control. Asterisks in (b) denote significant differences from the solvent control ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, according to χ^2 test) (data from Oehlmann *et al.*[9]).

DES stimulated a permanent increase in prostate size in male offspring, while at 20 μg/kg body wt/day no difference from the control was observed, and at 200 μg/kg body wt/day, a significant decrease in prostate size was observed (Figure 3). Follow-up studies have shown that there are structural differences between the control and 20 μg/kg body wt/day exposed prostate glands. There is also a marked increase in prostate size and hyperplasia of the glandular epithelium at low doses of DES, and a marked suppression of gland development at the 200 μg/kg body wt/day dose.[20] The low versus high dose findings for DES have also been reported by Gupta in both *in vivo* and *in vitro* experiments.[19] Maternal administration of very low doses of BPA (2.0–50 μg/kg body wt/day) also caused an identical permanent stimulation of the prostate in male mouse offspring, associated with a permanent upregulation of prostate androgen receptors.[14,19] Maternal administration of a low dose of BPA (25 μg/kg body wt/day) also stimulates a similar permanent increase in mammary gland ducts in female mouse offspring.[21] Many other inverted-U dose–response curves for BPA, DES and other endocrine-disrupting chemicals have been reported. There are over 100 published studies involving the use of low doses of BPA, including many showing inverted-U dose–response curves.[6] A document containing references to these studies and other information about BPA is available at http://rcp.missouri.edu/endocrinedisruptors/vomsaal/vomsaal.html. None of the reported low-dose effects of BPA can be considered beneficial.

It is difficult to imagine anyone proposing that the programming of the prostate to show hyperplasia would ever be desirable, as benign prostate hyperplasia can result in urethral obstruction and ultimately death if untreated in men. Furthermore, in mice, in addition to prostate enlargement, fetal exposure to manmade xenoestrogens such as BPA and DES results in multiple malformations of the urethra, including a marked constriction at the bladder neck.[20] The dose range of BPA that produces these effects in mice results in blood levels of unconjugated BPA that are within and even below the range of blood levels measured in human adults and fetuses.[22,23] Thus, adverse effects in mice occur at human exposure levels to BPA and at doses far below the dose predicted to be safe for humans.[24]

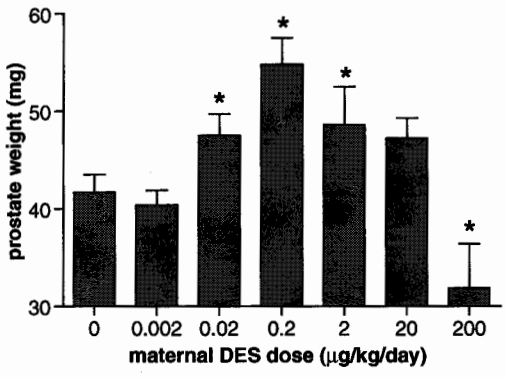

Figure 3 Prostate weight (mg) of male mouse offspring (*Mus musculus domesticus*) versus maternally administered DES dose (μg/kg body wt/day). Symbols are means with standard error. Asterisks denote significant differences from the control (*$P < 0.05$, according to LSmeans test following one-way ANOVA) (data from vom Saal *et al.*[18]).

Discussion

For the untrained observer, confusion between hormetic responses and low-dose effects of endocrine-disrupting chemicals, such as environmental oestrogens, is quite likely. However, the mechanism of action of the manmade xenoestrogens described above clearly distinguishes our examples from other types of hormetic responses for which mechanisms are unknown. Endocrine disruptors are defined by their mechanism, namely the type of interference with some aspect of the endocrine system, which includes all intercellular and even autocrine signalling systems. Whereas hormetic responses involve stimulation and are always higher than those of the control (other than perhaps studies of disease frequency), responses to low doses of endocrine disruptors may either be increased or decreased as compared to those of a control group, depending on the specific action of oestrogen in the tissue. Therefore, the confusion typically arises in cases of oestrogen responses that are stimulatory. Hormesis is regarded as an adaptive and by some proponents a beneficial phenomenon, because it is considered to result from stimulation of protective mechanisms.[2] In contrast, we are not aware of responses to manmade xenoestrogens encountered in the environment that would be considered beneficial, as they require energy that was not allocated for a particular process in the first place (for tissues where stimulatory effects occur, such as in the oviducts) or they disrupt organ function (for example, the testes). Consider the following cases of oestrogenic stimulation:

1. Oestrogenic chemical exposure of males in or out of the breeding season may lead to feminization, intersexuality and reduced sperm quality.[13,14,25,26]

2. Oestrogenic chemical exposure of females out of the breeding season leads to a stimulation of reproduction, which ultimately may cause a rupture of the oviduct as has been shown for prosobranch snails.[9] Furthermore, this stimulation is likely to

cause energy shortages in growth, maintenance and reserves. When exposure occurs out of season, females are unlikely to find a partner to fertilize them, and if, nevertheless, offspring are produced, they will encounter unfavourable circumstances in the outside world (e.g., suboptimal temperatures, lack of food and hiding places).[27,28] Oestrogenic chemical exposure of females in the breeding season may seem the most innocent case, but it could in fact lead to a reduced reproductive performance, which ultimately reduces the number of offspring during the most favourable time for juvenile growth and survival in the environment.[29]

3. In mammals, disruption of reproductive processes in offspring can occur following maternal exposure, such as abnormal rates of postnatal growth,[30] and changes in adult neuroendocrine and reproductive organ function.[31–33] Developmental exposure to very low doses of manmade xenoestrogens, such as BPA, can lead to early puberty,[30] and thus pregnancy during a time in life when fetuses are competing with the growing mother, resulting in a suboptimal pregnancy, suboptimal phenotype of offspring and an increase in mortality.[34]

From these examples it should be clear that effects of manmade xenoestrogens cannot be considered to be beneficial to the organism when many outcomes are examined and long-term consequences are considered, unlike what has been described for hormesis.[2] However, it should be noted that hormetic responses require energy as well, and might therefore induce energy shortages in the same way as manmade xenoestrogens. Also, what may appear to be a short-term advantage of a hormetic response on an isolated system could have adverse consequences over the long term, such as reduced lifespan or increased likelihood of disease in other systems that were not examined. The latter possibility was also acknowledged by Calabrese and Baldwin.[1] Consequently, many supposed examples of hormesis that have been proposed to be beneficial may not be when all long-term consequences are considered, and this should be considered in future investigations (e.g., by studying multiple endpoints and long-term effects).

What the two phenomena clearly have in common is the inverted U-shape type of the dose–response curve (see Figure 1a, 2a and 3), which is described as follows: at low concentrations a stimulated performance or response is evident (performance is higher than that of the control), which disappears at higher concentrations (performance is equal to that of the control), and eventually changes to inhibition (performance is lower than that of the control). For xenoestrogens, high dose inhibition can occur due to interference with an increasing number of endocrine-response systems as dose increases (e.g., due to binding or cross-talk of a xenoestrogen with other nuclear receptors), activation or inhibition of different genes at different doses,[35] and because at increasing concentrations all chemicals, including endogenous hormones, eventually reach toxic levels that will inhibit performance.[5]

A critical aspect of the findings presented here is that they demonstrate that low-dose stimulatory effects of manmade xenoestrogens cannot be viewed by regulatory agencies as typically beneficial. In contrast, Calabrese and Baldwin[2] proposed that hormesis should drive a paradigm shift, based on the view that the public has been unnecessarily 'frightened' by current assumptions underlying risk assessment. Instead of protecting against low-dose exposure, these authors proposed that the fear that there was no safe exposure dose for manmade chemicals should be counterbalanced,[2] based on the recognition that the beneficial low-dose effects of chemicals have been ignored.

It is important to emphasize that we are in complete agreement with the view that the inverted-U functions identified as hormesis,[2] and which have also been shown for octylphenol, BPA and DES above, should drive a paradigm shift in risk assessment. This is based on overwhelming evidence from decades of research on hormones and hormone-mimicking chemicals and drugs that: 1) linear extrapolation from experiments using only high doses cannot be used to predict effects at low doses;[5] and 2) at the receptor level there can be no threshold for chemicals that act via the same mechanism as endogenous hormones such as oestradiol, because endogenous oestradiol is already above the threshold level of activity in the organism.[36] Because these findings falsify the basic assumptions underlying risk assessment for noncarcinogenic chemicals (systemic toxicants), risk assessment as currently conducted using the threshold dose-response model cannot be considered as a science-based process.[37]

While we believe that the findings regarding hormesis and endocrine disruption both show that the threshold dose-response model used in current risk assessment has to be abandoned, we draw the opposite conclusion from Calabrese and Baldwin.[2] We propose that with regard to the published findings for endocrine disruptors, the threshold dose-response model will dramatically *underestimate risk* rather than overestimate risk for adverse effects at low doses, which is discussed in detail by Welshons *et al.*[5] As an example, there are over 30

published studies reporting a wide range of adverse effects at doses of BPA below the current reference dose of 50 μg/kg body wt/day (see vom Saal and Hughes,[6] and references at http://rcp.missouri.edu/endocrinedisruptors/vomsaal/vomsaal.html), which the public is assured is a dose at least 100-fold lower than that which could cause any effects based on the threshold dose-response model.[24]

Conclusions

By examining the mode-of-action, our aim has been to clear up confusion between hormesis and responses to low doses of manmade xenoestrogens. The stimulation of performance by manmade xenoestrogens cannot be viewed as the result of an over-reacting defence mechanism as in the case of hormesis. Therefore, manmade xenoestrogens should receive special treatment in risk assessment, taking care that even very low concentrations may cause responses deviating from the normal status. Such deviations result in impaired performance, and reduced fitness, because they require an allocation of energy or cause disruption of homeostatic systems that consequently will result in adverse outcomes. These energy shortages and other reductions in fitness may only become apparent when multiple parameters and endpoints, including long-latency outcomes, are determined in a bioassay. We strongly emphasize that the hormesis phenomenon (inverted-U dose–response curves) deserves attention with regard to the current threshold dose-response model used in risk assessment. However, the view of hormesis as an adaptive response should not be confused with adverse stimulatory responses induced by low doses of manmade xenoestrogens or other endocrine-disrupting chemicals.

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References

1 Calabrese EJ, Baldwin LA. Defining hormesis. *Hum Exp Toxicol* 2002; **21**: 91–97.
2 Calabrese EJ, Baldwin LA. Toxicology rethinks its central belief: hormesis demands a reappraisal of the way risks are assessed. *Nature* 2003; **421**: 691–92.
3 Kaiser J. Sipping from a poisoned chalice. *Science* 2003; **302**: 376–78.
4 Stebbing ARD. Hormesis – the stimulation of growth by low levels of inhibitors. *Sci Total Environ* 1982; **22**: 213–34.
5 Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* 2003; **111**: 994–1006.
6 vom Saal FS, Hughes C. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect* 2005; **113**: 926–33.
7 Duft M, Schulte-Oehlmann U, Weltje L, Tillmann M, Oehlmann J. Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail Potamopyrgus antipodarum. *Aquat Toxicol* 2003; **64**: 437–49.
8 Jobling S, Casey D, Rodgers-Gray T, Oehlmann J, Schulte-Oehlmann U, Pawlowski S, Braunbeck T, Turner AP, Tyler CR. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquat Toxicol* 2004; **66**: 207–22.
9 Oehlmann J, Schulte-Oehlmann U, Tillmann M, Markert B. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol as xeno-estrogens. *Ecotoxicology* 2000; **9**: 383–97.
10 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* 1996; **104**: 1296–300.
11 Alworth LC, Howdeshell KL, Ruhlen RL, Day JK, Huang H-M, Besch Williford C, Lubahn DB, vom Saal FS. Imprinting of uterine response to estradiol and ribosomal gene methylation due to fetal exposure to diethylstilbestrol and methoxychlor in CD-1 mice: opposite effects of low and high doses. *Toxicol Appl Pharmacol* 2002; **183**: 10–22.
12 Newbold RR, Jefferson WN, Padilla-Banks E, Haseman J. Developmental exposure to diethylstilbestrol (DES) alters uterine response to estrogens in prepubescent mice: low versus high dose effects. *Reprod Toxicol* 2004; **18**: 399–406.
13 Sakaue M, Ohsako S, Ishimura R, Kurosawa S, Kurohmaru M, Hayashi Y, Aoki Y, Yonemoto J, Tohyama C. Bisphenol A affects spermatogenesis in the adult rat even at a low dose. *J Occup Health* 2001; **43**: 185–90.
14 vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, Parmigiani S, Welshons WV. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol Ind Health* 1998; **14**: 239–60.
15 Hood TE, Calabrese EJ, Zuckerman BM. Detection of an estrogen receptor in two nematode species and inhibi-

tion of binding and development by environmental chemicals. *Ecotoxicol Environ Saf* 2000; **47**: 74–81.
16 Kooijman SALM. *Dynamic energy and mass budgets in biological systems*, second edition. Cambridge, UK: Cambridge University Press, 2000.
17 Oehlmann J, Schulte-Oehlmann U, Bachmann J, Oetken M, Lutz I, Kloas W, Ternes TA. Bisphenol A induces superfeminization in the ramshorn snail Marisa cornuarietis (Gastropoda: Prosobranchia) at environmentally relevant concentrations. *Environ Health Perspect* 2005; in press.
18 vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc Natl Acad Sci USA* 1997; **94**: 2056–61.
19 Gupta C. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc Soc Exp Biol Med* 2000; **224**: 61–68.
20 Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. *Proc Natl Acad Sci USA* 2005, **102**: 7014–19.
21 Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 2001; **65**: 1215–23.
22 Schönfelder G, Wittfoht W, Hopp H, Talsness C, Paul M, Chahoud I. Parent bisphenol A accumulation in human maternal-fetal-placental unit. *Environ Health Perspect* 2002; **110**: A703–707.
23 Takeuchi T, Tsutsumi O. Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. *Biochem Biophys Res Commun* 2002; **291**: 76–78.
24 IRIS. 2004. Bisphenol A. (CASRN 80-05-7). US-EPA Integrated Risk Information System (IRIS) Substance. Retrieved December, 2004, from: http://www.epa.gov/iris/subst/0356.htm.
25 Levy G, Lutz I, Kruger A, Kloas W. Bisphenol A induces feminization in Xenopus laevis tadpoles. *Environ Res* 2004; **94**: 102–11.
26 Talsness C, Fialkowski O, Gericke C, Merker H-J, Chahoud I. The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. *Congenit Anom* 2000; **40**: S94–107.
27 Andersen HR, Halling-Sorensen B, Kusk KO. A parameter for detecting estrogenic exposure in the copepod Acartia tonsa. *Ecotoxicol Environ Saf* 1999; **44**: 56–61.
28 Giesy JP, Pierens SL, Snyder EM, Miles-Richardson S, Kramer VJ, Snyder SA, Nichols, KM, Villeneuve DA. Effects of 4-nonylphenol on fecundity and biomarkers of estrogenicity in fathead minnows (Pimephales promelas). *Environ Toxicol Chem* 2000; **19**: 1368–77.
29 Schulte-Oehlmann U, Tillmann M, Casey D, Duft M, Markert B, Oehlmann J. Xeno-estrogenic effects of bisphenol A in prosobranch snails (Mollusca: Gastropoda: Prosobranchia). *Umwelt-wissenschaften und Schadstoff-Forschung Zeitschrift für Umweltchemie und Ökotoxikologie* 2001; **13**: 319–33.
30 Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. Exposure to bisphenol A advances puberty. *Nature* 1999; **401**: 763–64.
31 Rubin BS, Murray MK, Bamassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ Health Perspect* 2001; **109**: 657–80.
32 Schönfelder G, Flick B, Mayr L, Talsness C, Paul M, Chahoud I. In utero exposure to low doses of bisphenol A lead to long-term deleterious effects in the vagina. *Neoplasia* 2002; **4**: 98–102.
33 Steinmetz R, Brown NG, Allen DL, Bigsby RM, Ben-Jonathan N. The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. *Endocrinology* 1997; **138**: 1780–86.
34 Wang H-S, vom Saal FS. Maternal age influences traits of offspring. *Nature* 1999; **407**: 469–70.
35 Coser KR, Chesnes J, Hur J, Ray S, Isselbacher KJ, Shioda T. Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF7/BUS breast cancer cells by DNA microarray. *Proc Natl Acad Sci USA* 2003; **100**: 13994–99.
36 Sheehan DM, Willingham E, Gaylor D, Bergeron JM, Crews D. No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? *Environ Health Perspect* 1999; **107**: 155–59.
37 vom Saal FS, Sheehan DM. Challenging risk assessment. *Forum Appl Res Public Policy* 1998; **13**: 11–18.