



## Letter to the Editor

### Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: A response to attempts to mislead the public

Bisphenol A is the chemical monomer used in the manufacture of resins that line cans, polycarbonate plastic (including many food and beverage containers), and as an additive in many other products. We respond here to an article published in *Toxicology* (202:1, 2004) entitled: “Fraud, errors and gamesmanship in experimental toxicology” by Dr. Iain Purchase in which he criticized research we had conducted on bisphenol A, a chemical that shows oestrogenic activity. Those who read the article by Dr. Purchase were led to believe that no studies had been published that confirmed our seminal publication concerning oestrogenic effects of very low doses of bisphenol A (Nagel et al., 1997). The presumed absence of any replications (by us or anyone else) of our low-dose study of bisphenol A led Dr. Purchase to identify our discussions of these findings as a form of “gamesmanship”. In reality, our article was the first of a very large number of published studies showing that bisphenol A has a wide range of adverse effects below the dose predicted to be safe for humans (the reference dose or tolerable daily intake).

In the initial article by Nagel et al. (1997), we calculated that in vivo oestrogenic activity of bisphenol A in fetal mice would be detected at a 20  $\mu\text{g}/\text{kg}/\text{day}$  dose of bisphenol A. This prediction had major public health implications in that the prediction was that bisphenol A should exhibit biological activity below the reference dose of 50  $\mu\text{g}/\text{kg}/\text{day}$ . The prediction was based on:

1. our prior demonstration of very high potency of oestrogens during fetal development (vom Saal et al., 1997), and
2. our finding of limited binding of bisphenol A to oestrogen-binding proteins in human plasma, which results in a markedly elevated free plasma concentration of bisphenol A relative to oestradiol (Reviewed in: Nagel et al., 1999).

Our calculations (that were described in detail in the initial article by Nagel) led us to propose that this very low (and presumably safe) maternal dose of bisphenol A would produce a change in development of the reproductive system (including a permanently enlarged prostate) in fetal mice that would be similar to effects reported in response to a very small increase in the endogenous hormone oestradiol and very low maternal doses of the potent oestrogenic drugs, diethylstilbestrol (DES) and ethinyloestradiol. In our initial test of this hypothesis, we focused on the effects of bisphenol A on the developing prostate and testicular sperm production in males and determined that bisphenol A at 2–20  $\mu\text{g}/\text{kg}/\text{day}$  caused effects similar to oestradiol, DES and ethinyloestradiol (vom Saal et al., 1997, 1998; Thayer et al., 2001). We then published a study in *Nature* in which we reported that feeding pregnant mice a very low dose (2.4  $\mu\text{g}/\text{kg}/\text{day}$ ) of bisphenol A increased the rate of postnatal growth in male and female offspring and advanced puberty in female offspring (Howdeshell et al., 1999). In another study we showed that a 10  $\mu\text{g}/\text{kg}/\text{day}$  dose of bisphenol A decreased maternal behaviour in mice (Palanza et al., 2002). We have also conducted a detailed morphological and histochemical examination showing significant effects of low doses of bisphenol A, DES and ethinyloestradiol on the fetal prostate in mice, which we will describe in more detail below (Timms et al., 2005).

DOIs of original articles: [10.1016/j.tox.2005.05.005](https://doi.org/10.1016/j.tox.2005.05.005),  
[10.1016/j.tox.2005.05.004](https://doi.org/10.1016/j.tox.2005.05.004).

In addition, we determined that the rate of leaching of bisphenol A into water at room temperature from old, visibly worn polycarbonate animal cages is 1000-fold greater than the rate of leaching from new cages (Howdeshell et al., 2003), suggesting that the continued use of polycarbonate food or beverage products after they show evidence of wear can result in very high levels of exposure.

Out of a total of 125 published studies with low doses of bisphenol A that we accessed via a PubMed search in early March 2005, there have been 104 published studies reporting *in vivo* oestrogenic activity of bisphenol A at doses below the prior LOAEL of 50 mg/kg/day that was used to calculate the current reference dose in the USA of 50 µg/kg/day (IRIS, 1988). The US National Toxicology Program (NTP) categorizes these as “low-dose” studies (NTP, 2001). Low-dose effects refer to biological changes that occur in the range of human exposures or at doses that are lower than those typically used in the US-EPA standard testing paradigm for evaluating reproductive and developmental toxicity (Reviewed in: vom Saal and Welshons, 2000). Of the 104 low-dose studies reporting significant effects, there are a total of 38 published studies that report effects caused by doses of bisphenol A at and below the reference dose of 50 µg/kg/day. A list of these publications and their findings in invertebrates, fish, frogs, birds and mammals, as well as information on mechanisms of action from *in vitro* studies, pharmacokinetics, sources of exposure, and blood and tissue levels in animal and human studies, can be accessed at: <http://rcp.missouri.edu/endocrinedisruptors/vomsaal/vomsaal.html>.

Examples of effects caused by exposure to low doses of bisphenol A in rats and mice are a significant decrease in daily sperm production and fertility in male rats and mice at doses between 0.2 and 20 µg/kg/day (vom Saal et al., 1998; Sakaue et al., 2001; Al-Hiyasat et al., 2002; Chitra et al., 2003). In female mice there is significant disruption of the alignment of chromosomes during meiosis in developing oocytes due to leaching of bisphenol A from polycarbonate drinking bottles at doses between 15 and 70 µg/kg/day (Hunt et al., 2003), and an increase in mortality of embryos in mice at a maternal dose of 25 µg/kg/day (Al-Hiyasat et al., 2004). Fetal exposure (via the mother) to bisphenol A results in an early onset of sexual maturation in female mice at 2.4–20 µg/kg/day (Howdeshell

et al., 1999; Honma et al., 2002) and in another study at 500 (µg/kg/day, which was the lowest dose examined (Nikaïdo et al., 2004).

Gestational and lactational exposure to bisphenol A disrupts adult oestrous cycles in mice and rats at doses between 100 and 500 µg/kg/day (Talsness et al., 2000; Nikaïdo et al., 2004), and increases postnatal growth in both male and female mice and rats at maternal doses between 2.4 and 500 µg/kg/day (Howdeshell et al., 1999; Takai et al., 2000; Rubin et al., 2001; Nikaïdo et al., 2004). Neurochemical effects include stimulation in the brain of an increase in progesterone receptor mRNA levels at 400 µg/kg/day (Funabashi et al., 2003), as well as oestrogen receptor alpha (ERα) levels at 40 µg/kg/day (Aloisi et al., 2001), and oestrogen receptor beta (ERβ) mRNA at 25 µg/kg/day (Ramos et al., 2003). Bisphenol A also causes a change in brain somatostatin receptors at the lowest dose examined, which was 400 µg/kg/day (Facciolo et al., 2002). Behavioural effects include hyperactivity at 30 µg/kg/day (Ishido et al., 2004), an increase in aggressiveness at 2–40 µg/kg/day (Farabollini et al., 2002; Kawai et al., 2003), altered reactivity to painful or fear-provoking stimuli at 40 µg/kg/day (Aloisi et al., 2002), and impaired learning at 100 µg/kg/day (Negishi et al., 2004). Developmental exposure to bisphenol A also results in a significant change in the locus coeruleus, where bisphenol A at 30 µg/kg/day had the interesting effect of reversing the normal sex differences in this brain structure and eliminated sex differences in behaviour (Kubo et al., 2003). Other behaviours impacted by developmental exposure to low doses of bisphenol A are maternal behaviour at 10 µg/kg/day (Palanza et al., 2002), play and other socio-sexual behaviours at 40 µg/kg/day (Dessi-Fulgheri et al., 2002; Farabollini et al., 2002), rate of learning at 100 µg/kg/day (Negishi et al., 2004), and the behavioural response to drugs such as amphetamine at 40–300 µg/kg/day (Adriani et al., 2003; Suzuki et al., 2003). Bisphenol A also alters plasma luteinizing hormone (LH) levels at 2 µg/kg/day (Akingbemi et al., 2004) and decreases plasma testosterone in males at 2 µg/kg/day (Kawai et al., 2003; Akingbemi et al., 2004). Bisphenol A interferes with the immune system at 2.5–30 µg/kg/day (Sawai et al., 2003; Yoshino et al., 2003, 2004), as well as antioxidant enzymes at the very low dose of 0.2 µg/kg/day (Chitra et al., 2003).

About one-half of the low-dose bisphenol A studies have been published in just the last 2 years. A thorough analysis of the entire published low-dose bisphenol A literature by regulatory agencies that takes into account the issues discussed here and elsewhere (vom Saal and Hughes, 2005; vom Saal et al., 2005) is now warranted. The most recent risk assessment for bisphenol A was conducted by the European Union based on a review of the literature published as of 1998, although a few additional studies published after 1998 were also mentioned (Bureau, 2003).

While the above is not a comprehensive list, it is clear that there is an overwhelming published literature supporting our initial hypothesis that bisphenol A would have much greater oestrogenic activity *in vivo* than had been predicted based on prior traditional toxicological studies that had only examined a few very high doses (Morrissey et al., 1987). This fact stands in stark contrast to the statements about this issue made by Dr. Purchase, who included our publication as a case study in fraud with the title: “Case Study 8: Environmental oestrogens—are they really that potent?” Dr. Purchase stated that: “Dr. Frederick vom Saal, the corresponding author on the original Nagel paper, continues to advocate that the effects of bisphenol A and other oestrogenic chemicals occur at low doses and that there is an inverted dose–response relationship at these low doses.” [In fact, Dr. Welshons was the corresponding author for this article.] Dr. Purchase went on to state: “He [Dr. vom Saal] has failed to publish any evidence or to demonstrate that the result is reproducible” (p. 19). “Advocating that effects are seen at low doses, when there is no evidence of its reproducibility and indeed evidence that it is not reproducible in other laboratories is a form of gamesmanship” (p. 8). These statements by Dr. Purchase are clearly not consistent with the facts reported above.

Dr. Purchase also stated in his article: “vom Saal has reported on prostate weight in mice at very low doses; no such effects have been seen by others” (p. 10). Verification through *independent* replication is the criterion for acceptance of published findings as valid, and fraudulent research is unlikely to be validated. In contrast to the statement by Dr. Purchase, our specific findings were validated in a replication of our study in which 50  $\mu\text{g}/\text{kg}/\text{day}$  bisphenol A and 0.1  $\mu\text{g}/\text{kg}/\text{day}$  DES (as a positive control) were fed to pregnant CD-1 mice, and a significant increase in prostate size and

prostate androgen receptors was observed in male offspring for both chemicals at three different postnatal ages (Gupta, 2000). In the same article Gupta reported that in primary culture, the fetal mouse prostate showed an increase in size and prostate ducts in response to DES at a dose of 0.1 pg/ml (0.1 parts per trillion or ppt) and to bisphenol A at a dose of 50 pg/ml (50 ppt) serum-free culture medium. It is important to note that the scientist who conducted this successful replication is a highly regarded reproductive endocrinologist with over 25 years of publications concerning effects of hormones and chemicals on development of the reproductive system in male rats and mice.

Dr. Purchase repeatedly implied that replication by us, rather than independent replication of our initial finding regarding stimulation of growth of the fetal prostate by bisphenol A, was the criterion for acceptance of these results as reliable by the scientific community. In fact, in a detailed follow-up to our initial finding and the results reported by Gupta, we found that a maternal bisphenol A oral dose of 10  $\mu\text{g}/\text{kg}/\text{day}$  during the last 5 days of pregnancy in CD-1 mice significantly increased the number of primary prostate ducts and the rate of proliferation of the primary duct epithelium, resulting in the volume of the prostate being increased by almost two fold relative to controls on gestation day 19, 2 days after the initiation of prostate duct development in male fetuses. This low dose of bisphenol A also resulted in multiple malformations of the urethra, including a marked constriction at the bladder neck, and all of these effects were observed in response to both DES and ethinyloestradiol at a dose of 0.1  $\mu\text{g}/\text{kg}/\text{day}$  (Timms et al., 2005). In a related study, female CD-1 mice responded to a very low maternal bisphenol A dose (administered by Alzet pump) of 0.025  $\mu\text{g}/\text{kg}/\text{day}$  by showing a permanent increase in mammary gland ducts (Markey et al., 2001a).

In addition to these findings in mice, stimulation of proliferation of cultured human prostate cancer cells by bisphenol A was reported at a dose of 23 ppt culture medium, maximal proliferation occurred at 230 ppt, while no response was observed at 23 parts per billion (ppb) (Wetherill et al., 2002). In contradiction to the statement by Dr. Purchase about the issue of inverted-U dose–response curves, there are 16 published examples of inverted-U dose–response curves for bisphenol A (see website above and the review by Welshons et al., 2003). The presence of inverted-U

dose–response curves showing unique effects at doses far below the assumed no effect dose based on studies that only examined a few very high doses of bisphenol A has profound implications for risk assessment. We discuss in detail elsewhere that the presence of inverted-U dose–response curves invalidates the linear-threshold model used in chemical risk assessment to calculate “safe” doses for human exposure (vom Saal and Sheehan, 1998; Welshons et al., 2003; vom Saal and Hughes, 2005).

In his article in *Toxicology*, Dr. Purchase’s review of the state of research on low-level effects of bisphenol A discusses only three published papers: our original publication and two failed efforts at replication funded by chemical corporations (Ashby et al., 1999; Cagen et al., 1999). However, there are 104 published studies showing significant effects at low doses of bisphenol A previously assumed to cause no effect (some of these are reviewed above). We are aware of 21 published studies reporting no effects at doses of bisphenol A below the prior LOAEL of 50 mg/kg/day. It is thus important to examine the factors associated with these reports of negative results for low doses of bisphenol A.

Factor 1: Nine out of the 21 studies reporting only negative results involved the use of the Charles River Sprague–Dawley (CD-SD) rat, and no study that used this rat reported positive effects of low doses of bisphenol A. Studies have shown that this rat strain has a low sensitivity to exogenous oestrogenic chemicals after over 40 years of selective breeding (for rapid growth and large litter size) of rats initially purchased from Sprague–Dawley by Charles River Laboratories. Specifically, the CD-SD rat required a high dose of ethinyloestradiol (50–200  $\mu\text{g}/\text{kg}/\text{day}$ ), which was included as a positive control, to show most responses in a study in which no low-dose effects of bisphenol A were found (Yamasaki et al., 2002); the typical dose of ethinyloestradiol in oral contraceptives is about 0.5  $\mu\text{g}/\text{kg}/\text{day}$  for a 60 kg woman. It would be inappropriate to cite this article by Yamasaki as evidence of the absence of low-dose effects of bisphenol A without reporting that the findings for the positive control, ethinyloestradiol, reveal the very low sensitivity of this model animal to any exogenous oestrogen. Failure to find effects of low doses of bisphenol A in another large study funded by the chemical industry would likely have been shown to be due to the very low sensitiv-

ity to exogenous oestrogens of the CD-SD rat that was used if the study had included a positive control such as ethinyloestradiol in the experimental design (Tyl et al., 2002).

The study by Tyl et al. (2002) was specifically criticized by a panel convened by the US National Toxicology Program to examine the issue of low-dose effects of endocrine disrupting chemicals. The Low Dose NIH Panel noted in the executive summary of the published report that: “In selecting animal models for study, the Panel advocated the use of species and strains that are highly responsive to endocrine active agents of concern (i.e. responsive to positive controls), not on convenience and familiarity” (NTP, 2001). The very low sensitivity of the CD-SD rat to ethinyloestradiol contrasts with significant effects that were produced in male CF-1 mice that we used in our initial experiments at the much lower maternal ethinyloestradiol oral dose of 0.002  $\mu\text{g}/\text{kg}/\text{day}$  (Thayer et al., 2001); it is thus not surprising that effects similar to those caused by ethinyloestradiol were observed in male offspring in response to 2–20  $\mu\text{g}/\text{kg}/\text{day}$  bisphenol A fed to pregnant CF-1 mice (vom Saal et al., 1998).

Factor 2: Dr. Purchase does not mention that one of the failed replication studies he cited (Ashby et al., 1999) was previously criticized by us (ENDS, 1998) and by the Low Dose NIH Panel for errors in interpreting the data (NTP, 2001). In fact, Dr. Purchase was director of the ICI/Zeneca Central Toxicology Laboratory in Macclesfield from 1981 to 1998 (IUTOX, 2004), where he directed the laboratory in which this controversial research on bisphenol A was conducted. Readers should have been made aware at the beginning of the article about Dr. Purchase’s prior employment by ICI/Zeneca and involvement in one of the studies cited as the basis for criticizing our research.

Importantly, both of the industry-funded studies cited by Dr. Purchase (Ashby et al., 1999; Cagen et al., 1999) failed to accurately identify that the drug DES was included as a positive control rather than just another test chemical. In the initial report on the replication by Cagen et al., issued at a press conference and in a press release (Toloken, 1998), industry spokesmen reported that DES was included as a positive control and that the research found no effects of either the positive control (DES) or the test chemical (bisphenol A). The failure to find a difference between the negative controls and the positive controls on any mea-

sure should have led those involved in these studies to conclude that some factor had rendered their animals insensitive to even a highly potent oestrogenic drug. Indeed the NIH Low Dose Panel noted that regarding “a study in which the positive control does not produce the expected positive response. The prudent course of action in such cases may be to declare the study inadequate and repeat it, regardless of the experimental outcome in the test groups.” (NTP, 2001) (pp. 5–10).

Throughout Dr. Purchase’s article he repeatedly suggests that risk assessments should only use information from studies conducted by chemical corporations using good laboratory practices (GLP). This proposal is based on the assumption that GLP means that all aspects of the research will be free of bias. Failure of researchers to acknowledge to reviewers or the scientific community that the positive control chemical failed to show the expected positive effects is just one example of how bias can impact the interpretation of research.

Factor 3: Seven articles have been published that show a markedly lower sensitivity of the rat and mouse uterus to bisphenol A relative to other oestrogenic chemicals, such as DES, in what is generally regarded as the “gold standard” test in toxicology for oestrogenic activity, the uterotrophic assay (see website above for references). In prepubertal CD-1 mice, for example, a dose of 100 mg/kg/day that was injected sc was required to stimulate an increase in uterine weight (Markey et al., 2001b). No uterotrophic response to 7 mg/kg/day bisphenol A (the highest dose examined) administered tonically via a Silastic implant in neonatal CD-1 female mice was observed, while a uterotrophic response to 0.01 µg/kg/day DES occurred (Howdeshell, 2002). This is in marked contrast to the fetal CD-1 mouse urogenital sinus, which showed significant effects of maternal oral administration of 0.1 µg/kg/day DES that were identical to effects of oral administration of 10 µg/kg/day bisphenol A (Timms et al., 2005). Only by examining the uterus using much more sophisticated methods than just measuring uterine weight are effects of low doses observed (Markey et al., 2001b; Newbold et al., 2004).

In contrast to the very low sensitivity of the uterotrophic response as a bioassay to reveal the high in vivo potency of bisphenol A, in the CD-1 mouse strain that we now use in our experiments, long-

term effects observed during postnatal life due to developmental exposure to doses between 0.025 and 50 µg/kg/day (at and below the current reference dose) are: increased number of prostate glands and prostate volume (Gupta, 2000; Timms et al., 2005), increased aggression, decreased testis weight and decreased serum testosterone (Kawai et al., 2003), decreased maternal behaviour (Palanza et al., 2002), and stimulation of mammary glands, an increase in body weight and disruption of oestrous cycles (Markey et al., 2001a, 2003). Our decision to use the CD-1 mouse as a model to study low-dose effects of bisphenol A was based on the use of this mouse by the reproductive toxicology program within the US-NTP to study xeno-oestrogens (Newbold et al., 2004).

Taken together, the findings described above are consistent with the view that the action of oestrogenic chemicals such as bisphenol A in different tissues varies as a function of receptor sub-type (alpha or beta) as well as the co-regulators expressed in the tissue (Routhledge et al., 2000). In addition, the significant variability in the sensitivity of animal models to bisphenol A has to be taken into account in designing experiments. Recent reports that bisphenol A causes a variety of effects in cells that are far to rapid (in seconds to a few minutes) for them to be mediated by the classical nuclear receptors also has to be taken into account in assessing whether low-dose effects of bisphenol A that are being reported are plausible. A variety of rapid, non-genomic effects are being reported at extremely low doses of oestradiol and xeno-oestrogens, including bisphenol A. For example, in rat pituitary tumor cells, bisphenol A significantly stimulated a rapid (within 30 s) influx of calcium at the lowest dose that was examined (0.23 ppt or  $10^{-12}$  M); the greatest response occurred at 230 ppt, while the magnitude of the response decreased at 2.3 ppb, forming an inverted-U dose–response curve. The calcium influx response to bisphenol A at 230 ppt was actually greater than that for oestradiol or DES. Prolactin release, which is triggered by calcium influx in these cells, was detected within 1 min at 0.23 ppt bisphenol A, similar to the response to oestradiol (Wozniak et al., 2005). Another response associated with rapid induction of calcium influx in mouse pancreatic  $\beta$  cells is activation (phosphorylation) of the transcription factor CREB at 230 ppt (1 nM) bisphenol A, which is equal in magnitude to the response caused by the same

dose of oestradiol (Quesada et al., 2002). In addition, rapid (within 1.5 min) influx of calcium was observed in human MCF-7 breast cancer cells in response to 0.1 nM oestradiol or bisphenol A that was significant at the lowest dose tested; for oestradiol the EC50 was 0.11 nM and for bisphenol A the EC50 was 0.15 nM or about 34 ppt (Walsh et al., 2005).

The extensive evidence that bisphenol A can disrupt mouse, rat and human cell function at low part per trillion doses, and that disruption at the same low doses is also found in snails (Schulte-Oehlmann et al., 2001), has profound implications for human health, since the range of unconjugated bisphenol A in human fetal blood was reported to be 0.2–9 ppb (mean = 2.9 ppb), the range in maternal blood at the end of pregnancy was 0.3–19 ppb (mean = 4.4 ppb) (Schonfelder et al., 2002), and levels in human fetal amniotic fluid during sexual differentiation between gestation week 15–18 were even higher, averaging 8 ppb (Ikezuki et al., 2002). That levels of bisphenol A in human blood are high enough to result in abnormalities similar to those observed in experimental animals is indicated by the results of a case-control epidemiological study comparing bisphenol A levels in non-obese and obese women in Japan who had normal ovarian function or polycystic ovarian disease. There were significantly higher blood levels of bisphenol A in both obese women and in women with polycystic ovarian disease. The lowest blood levels of bisphenol A occurred in non-obese women with normal ovaries (mean  $\pm$  SEM =  $0.71 \pm 0.09$  ppb), while the highest blood levels ( $1.17 \pm 0.16$  ppb) occurred in obese women with ovarian disease (Takeuchi et al., 2004).

The information presented here reveals that scientists are rapidly determining:

1. the mechanisms by which very low doses of bisphenol A can cause disruption of the endocrine mechanisms that regulate cell function,
2. the adverse health effects caused by exposure to very low doses of bisphenol A during different life stages in experimental animals,
3. the correlation between exposure to bisphenol A and diseases in humans in studies based on findings with experimental animals.

The endocrine disrupting effects caused by very low doses of chemicals such as bisphenol A that can interact with components of the endocrine system are funda-

mentally different from and not predicted by studies that only examine toxic effects caused by very high doses (Welshons et al., 2003). Receptors for oestrogenic chemicals, including the classical nuclear ligand-dependent transcription factors ER $\alpha$  and ER $\beta$ , and particularly the receptors associated with the cell membrane and involved in initiating signaling cascades leading to activation of kinases, evolved to provide great amplifying capability, resulting in large responses to extremely low doses of oestrogens (vom Saal et al., 1997; Welshons et al., 2003). The article by Dr. Purchase failed to acknowledge this reality and the extensive published low-dose literature on bisphenol A that is consistent with its ability to stimulate responses mediated by oestrogen receptors associated with specific genes in the nucleus or associated with the cell membrane. At the same time that the article appeared in *Toxicology* by Dr. Purchase, a report funded by the American Chemistry Council based on a review of only 19 carefully selected low-dose studies of bisphenol A also concluded that the evidence for low-dose effects of bisphenol A was weak (Gray et al., 2004). This report has been criticized as inaccurate, and initiation of a new risk assessment for bisphenol A based on the current published literature was proposed (vom Saal and Hughes, 2005).

Everyone in science is harmed when misinformation about a critical public health issue appears in scientific journals. When an article by someone such as Dr. Purchase with a long association with a chemical corporation contains false information, it not only further erodes the public's confidence in the ability of corporations to engage in basic science, but also has a negative impact on the public's perception regarding the credibility of all scientists. Only by being vigilant and taking swift action when there is clear evidence of misbehaviour can the scientific community hope to maintain the public's confidence.

## References

- Adriani, W., Della Seta, D., Dessi-Fulgheri, F., Farabollini, F., Laviola, G., 2003. Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. *Environ. Health Perspect.* 111, 395–401.
- Akingbemi, B.T., Sottas, C.M., Koulova, A.I., Klinefelter, G.R., Hardy, M.P., 2004. Inhibition of testicular steroidogenesis by

- the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology* 145, 592–603.
- Al-Hiyasat, A.S., Darmani, H., Elbetieha, A.M., 2002. Effects of bisphenol A on adult male mouse fertility. *Eur. J. Oral Sci.* 110, 163–167.
- Al-Hiyasat, A.S., Darmani, H., Elbetieha, A.M., 2004. Leached components from dental composites and their effects on fertility of female mice. *Eur. J. Oral Sci.* 112, 267–272.
- Aloisi, A.M., Della Seta, D., Ceccarelli, I., Farabollini, F., 2001. Bisphenol-A differently affects estrogen receptors- $\alpha$  in estrous-cycling and lactating female rats. *Neurosci. Lett.* 310, 49–52.
- Aloisi, A.M., Della Seta, D., Rendo, C., Ceccarelli, I., Scaramuzzino, A., Farabollini, F., 2002. Exposure to the estrogenic pollutant bisphenol A affects pain behavior induced by subcutaneous formalin injection in male and female rats. *Brain Res.* 937, 1–7.
- Ashby, J., Tinwell, H., Haseman, J., 1999. Lack of effects for low dose levels of bisphenol A (BPA) and diethylstilbestrol (DES) on the prostate gland of CF1 mice exposed in utero. *Reg Tox Pharm.* 30, 156–166.
- Bureau, E.C., 2003. Bisphenol A: European Union Risk Assessment Report (CAS No: 80-05-7) Volume 37. Luxembourg, Office for Official Publications of the European Communities.
- Cagen, S.Z., Waechter, J.M., Dimond, S.S., Breslin, W.J., Butala, J.H., Jekat, F.W., Joiner, R.L., Shiotsuka, R.N., Veenstra, G.E., Harris, L.R., 1999. Normal reproductive organ development in CF-1 mice following prenatal exposure to Bisphenol A. *Tox. Sci.* 11, 15–29.
- Chitra, K.C., Latchoumycandane, C., Mathur, P.P., 2003. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology* 185, 119–127.
- Dessi-Fulgheri, F., Porrini, S., Farabollini, F., 2002. Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. *Environ. Health Perspect.* 110 (Suppl. 3), 403–407.
- ENDS, 1998. Industry oestrogen study “fundamentally flawed”. The ENDS Report 285, pp. 4–5.
- Facciolo, R.M., Alo, R., Madeo, M., Canonaco, M., Dessi-Fulgheri, F., 2002. Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst2. *Environ. Health Perspect.* 110 (Suppl. 3), 397–402.
- Farabollini, F., Porrini, S., Della Seta, D., Bianchi, F., Dessi-Fulgheri, F., 2002. Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. *Environ. Health Perspect.* 110 (Suppl. 3), 409–414.
- Funabashi, T., Sano, A., Mitsushima, D., Kimura, F., 2003. Bisphenol A increases progesterone receptor immunoreactivity in the hypothalamus in a dose-dependent manner and affects sexual behaviour in adult ovariectomized rats. *J. Neuroendocrinol.* 15, 134–140.
- Gray, G.M., Cohen, J.T., Cunha, G., Hughes, C., McConnell, E.E., Rhomberg, L., Sipes, I.G., Mattison, D., 2004. Weight of the evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. *Hum. Ecol. Risk Assess.* 10, i875–i921.
- Gupta, C., 2000. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc. Soc. Exp. Biol. Med.* 224, 61–68.
- Honma, S., Suzuki, A., Buchanan, D.L., Katsu, Y., Watanabe, H., Iguchi, T., 2002. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod. Toxicol.* 16, 117–122.
- Howdeshell, K.L., 2002. Effects of exposure to environmentally relevant levels of bisphenol A on mouse reproductive physiology and maternal behavior, Unpublished PhD Thesis, University of Missouri-Columbia.
- Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenberg, J.G., vom Saal, F.S., 1999. Exposure to bisphenol A advances puberty. *Nature* 401, 763–764.
- Howdeshell, K.L., Peterman, P.H., Judy, B.M., Taylor, J.A., Orazio, C.E., Ruhlen, R.L., vom Saal, F.S., Welshons, W.V., 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. *Environ. Health Perspect.* 111, 1180–1187.
- Hunt, P.A., Koehler, K.E., Susiarjo, M., Hodges, C.A., Hagan, A., Voigt, R.C., Thomas, S., Thomas, B.F., Hassold, T.J., 2003. Bisphenol A causes meiotic aneuploidy in the female mouse. *Curr. Biol.* 13, 546–553.
- Ikezuki, U., Tsutsumi, O., Takai, Y., Kamei, Y., Taketani, Y., 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum. Reprod.* 17, 2839–2841.
- IRIS, 1988. Bisphenol A. (CASRN 80-05-7), US-EPA Integrated Risk Information System Substance file. <http://www.epa.gov/iris/subst/0356.htm>. Access date: 2 January 2005.
- Ishido, M., Masuo, Y., Kunimoto, M., Oka, S., Morita, M., 2004. Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. *J. Neurosci. Res.* 76, 423–433.
- IUTOX, 2004. 2004 Merit Award Winner, International Union of Toxicology, <http://www.iutox.org/meritaward2004.asp>. Access date: 2 January 2005.
- Kawai, K., Takehiro, N., Nishikata, H., Aou, S., Takii, M., Kubo, C., 2003. Aggressive behavior and serum testosterone concentration during the maturation process of male mice: The effects of fetal exposure to bisphenol A. *Environ. Health Perspect.* 111, 175–178.
- Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., Aou, S., 2003. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci. Res.* 45, 345–356.
- Markey, C.M., Coombs, M.A., Sonnenschein, C., Soto, A.M., 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. *Evol. Dev.* 5, 67–75.
- Markey, C.M., E.H. Luque, M. Munoz De Toro, C. Sonnenschein and A. M. Soto, 2001a. In utero exposure to bisphenol a alters the development and tissue organization of the mouse mammary gland. *Biol. Reprod.* 65, 1215–1223. Erratum correcting the dose administered was published in *Biol. Reprod.* 71:1753, 2004.
- Markey, C.M., Michaelson, C.L., Veson, E.C., Sonnenschein, C., Soto, A.M., 2001b. The mouse uterotrophic assay: a reevaluation.

- tion of its validity in assessing the estrogenicity of bisphenol A. *Environ. Health Perspect.* 109, 55–60.
- Morrissey, R.E., George, J.D., Price, C.J., Tyl, R.W., Marr, M.C., Kimmel, C.A., 1987. The developmental toxicity of bisphenol A in rats and mice. *Fund. Appl. Toxicol.* 8, 571–582.
- Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M., Welshons, W.V., 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ. Health Perspect.* 105, 70–76.
- Nagel, S.C., vom Saal, F.S., Welshons, W.V., 1999. Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. *J. Steroid Biochem. Mol. Biol.* 69, 343–357.
- Negishi, T., Kawasaki, K., Suzuki, S., Maeda, H., Ishii, Y., Kyuwa, S., Kuroda, Y., Yoshikawa, Y., 2004. Behavioral alterations in response to fear-provoking stimuli and tranlycypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. *Environ. Health Perspect.* 112, 1159–1164.
- Newbold, R.R., Jefferson, W.N., Padilla-Banks, E., Haseman, J., 2004. Developmental exposure to diethylstilbestrol (DES) alters uterine response to estrogens in prepubescent mice: low versus high dose effects. *Reprod. Toxicol.* 18, 399–406.
- Nikaido, Y., Yoshizawa, K., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., Tsubura, A., 2004. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod. Toxicol.* 18, 803–811.
- NTP, N.T.P., 2001. Final Report of the Endocrine Disruptors Low Dose Peer Review Panel. *Endocrine Disruptors Low Dose Peer Review*, Raleigh, NC, <http://ntp-server.niehs.nih.gov/hdocs/liason/LowDoseWebPage.html>. Access date: 2 January 2005.
- Palanza, P., Howdeshell, K.L., Parmigiani, S., vom Saal, F.S., 2002. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environ. Health Perspect.* 110, 415–422.
- Quesada, I., Fuentes, E., Viso-Leon, M.C., Soria, B., Ripoll, C., Nadal, A., 2002. Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *FASEB J.* 16, 1671–1673.
- Ramos, J.G., Varayoud, J., Kass, L., Rodriguez, H., Costabel, L., Munoz-De-Toro, M., Luque, E.H., 2003. Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. *Endocrinology* 144, 3206–3215.
- Routhledge, E.J., White, R., Parker, M.G., Sumpter, J.P., 2000. Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER)  $\alpha$  and ER $\beta$ . *J. Biol. Chem.* 275, 35986–35993.
- Rubin, B.S., Murray, M.K., Bamassa, D.A., King, J.C., Soto, A.M., 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ. Health Perspect.* 109, 657–680.
- Sakaue, M., Ohsako, S., Ishimura, R., Kurosawa, S., Kurohmaru, M., Hayashi, Y., Aoki, Y., Yonemoto, J., Tohyama, C., 2001. Bisphenol A affects spermatogenesis in the adult rat even at a low dose. *J. Occup. Health* 43, 185–190.
- Sawai, C., Anderson, K., Walsler-Kuntz, D., 2003. Effect of bisphenol A on murine immune function: Modification of interferon-gamma, IgG2a, and disease symptoms in NZB x NZW F1 mice. *Environ. Health Perspect.* 111, 1883–1887.
- Schonfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M., Chahoud, I., 2002. Parent bisphenol A accumulation in human maternal-fetal-placental unit. *Environ. Health Perspect.* 110, A703–A707.
- Schulte-Oehlmann, U., Tillmann, M., Casey, D., Duft, M., Markert, B., Oehlmann, J., 2001. Xeno-estrogenic effects of bisphenol A in prosobranchs (Mollusca: Gastropoda: prosbranchia). *Z. Umweltchem. Okotox.* 13, 319–333.
- Suzuki, T., Mizuo, K., Nakazawa, H., Funae, Y., Fushiki, S., Fukushima, S., Shirai, T., Narita, M., 2003. Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: enhancement of the methamphetamine-induced abuse state. *Neuroscience* 117, 639–644.
- Takai, Y., Tsutsumi, O., Ikezaki, Y., Kamei, Y., Osuga, Y., Yano, T., Taketan, Y., 2000. Preimplantation exposure to bisphenol A advances postnatal development. *Reprod. Toxicol.* 15, 71–74.
- Takeuchi, T., Tsutsumi, O., Ikezaki, Y., Takai, Y., Taketani, Y., 2004. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. *Endocr. J.* 51, 165–169.
- Talsness, C., Fialkowski, O., Gericke, C., Merker, H.-J., Chahoud, I., 2000. The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. *Congenital Anomalies* 40, 94–S107.
- Thayer, K.A., Ruhlen, R.L., Howdeshell, K.L., Buchanan, D., Cooke, P.S., Welshons, W.V., vom Saal, F.S., 2001. Altered reproductive organs in male mice exposed prenatally to sub-clinical doses of 17a-ethinyl estradiol. *Hum. Reprod.* 16, 988–996.
- Timms, B.G., Howdeshell, K.L., Barton, L., Bradley, S., Richter, C.A., vom Saal, F.S., 2005. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the mouse prostate and urethra. *Proc. Natl. Acad. Sci.* 102, 7014–7019.
- Toloken, S., 1998. SPI study disputes endocrine disruptor findings. *Plastic News* October 16.
- Tyl, R., Myers, C., Marr, M., Thomas, B., Keimowitz, A., Brine, D., Veselica, M., Fail, P., Chang, T., Seely, J., Joiner, R., Butala, J., Dimond, S., Cagen, S., Shiotsuka, R., Stropp, G., Waechter, J., 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol. Sci.* 68, 121–146.
- vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani, S., Welshons, W.V., 1998. 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* 14, 239–260.
- vom Saal, F.S., Hughes, C., 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ. Health Perspect.* Available online 13 April 2005.



- vom Saal, F.S., Richter, C.A., Ruhlen, R.L., Nagel, S.C., Timms, B.G., Welshons, W.V., 2005. Importance of appropriate controls, animal feed and animal models in interpreting results from low-dose studies of bisphenol A. *Birth Defects Res. Part A* 73, 140–145.
- vom Saal, F.S., Sheehan, D.M., 1998. Challenging risk assessment. *Forum Appl. Res. Public Pol.* 13, 11–18.
- vom Saal, F.S., Timms, B.G., Montano, M.M., Palanza, P., Thayer, K.A., Nagel, S.C., Dhar, M.D., Ganjam, V.K., Parmigiani, S., Welshons, W.V., 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc. Nat. Acad. Sci.* 94, 2056–2061.
- vom Saal, F.S., Welshons, W.V., 2000. NIH panel confirms that endocrine disrupting chemicals cause effects at very low doses. *Risk Pol. Rep.* 7, 47–50 (via Inside EPA.com, access date: 2 January 2005).
- Walsh, D.E., Dockery, P., Doolan, C.M., 2005. Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [CA<sup>++</sup>] in human breast cancer cells. *Mol. Cell Endocrinol.* 230, 23–30.
- Welshons, W.V., Thayer, K.A., Judy, B.M., Taylor, J.A., Curran, E.M., vom Saal, F.S., 2003. Large effects from small exposures I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ. Health Perspect.* 111, 904–1006.
- Wetherill, Y.B., Petra, C.E., Monk, K.R., Puga, A., Knudsen, K.E., 2002. The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostate adenocarcinoma cells. *Mol. Cancer Therapeut.* 7, 515–524.
- Wozniak, A.L., Bulayeva, N.N., Watson, C.S., 2005. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-a mediated Ca<sup>++</sup> fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ. Health Perspect.* On line, 14 January 2005.
- Yamasaki, K., Sawaki, M., Noda, S., Inmatanaka, N., Takatsuki, M., 2002. Subacute oral toxicity study of ethinylestradiol and bisphenol A, based on the draft protocol for the 'Enhanced OECD Test Guideline no 407'. *Arch. Toxicol.* 76, 65–74.
- Yoshino, S., Yamaki, K., Li, X., Sai, T., Yanagisawa, R., Takano, H., Taneda, S., Hayashi, H., Mori, Y., 2004. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. *Immunology* 112, 489–495.
- Yoshino, S., Yamaki, K., Yanagisawa, R., Takano, H., Hayashi, H., Mori, Y., 2003. Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. *Br. J. Pharmacol.* 138, 1271–1276.

Frederick S. vom Saal\*

Susan C. Nagel

*Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211, USA*

Barry G. Timms

*Division of Basic Biomedical Sciences, University of South Dakota, Vermillion, SD 57069, USA*

Wade V. Welshons

*Department of Biomedical Sciences, University of Missouri-Columbia, Columbia, MO 65211, USA*

\*Corresponding author. Tel.: +1 573 882 4367

fax: +1 573 884 5020

E-mail address: vomsaalf@missouri.edu

(F.S. vom Saal)

Available online 21 June 2005