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Review

Components of plastic: experimental studies in animals and relevance for human health

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Components used in plastics, such as phthalates, bisphenol A (BPA), polybrominated diphenyl ethers (PBDE) and tetrabromobisphenol A (TBBPA), are detected in humans. In addition to their utility in plastics, an inadvertent characteristic of these chemicals is the ability to alter the endocrine system. Phthalates function as anti-androgens while the main action attributed to BPA is oestrogen-like activity. PBDE and TBBPA have been shown to disrupt thyroid hormone homeostasis while PBDEs also exhibit anti-androgen action. Experimental investigations in animals indicate a wide variety of effects associated with exposure to these compounds, causing concern regarding potential risk to human health. For example, the spectrum of effects following perinatal exposure of male rats to phthalates has remarkable similarities to the testicular dysgenesis syndrome in humans. Concentrations of BPA in the foetal mouse within the range of unconjugated BPA levels observed in human foetal blood have produced effects in animal experiments. Finally, thyroid hormones are essential for normal neurological development and reproductive function. Human body burdens of these chemicals are detected with high prevalence, and concentrations in young children, a group particularly sensitive to exogenous insults, are typically higher, indicating the need to decrease exposure to these compounds.

Keywords: plastic; endocrine disruptor; phthalates; bisphenol A; polybrominated diphenyl ether; tetrabromobisphenol A

1. INTRODUCTION

Due to the high-volume generation of plastics and low production costs, consumers typically use many plastic items only once before discarding them. Environmental concerns associated with plastic use are not only because of the amount of waste, but also the leaching of substances out of the plastic. Components used in plastics, such as bisphenol A (BPA), polybrominated diphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA) and phthalates, are released from plastic products, and are also known as endocrine-disrupting compounds (EDCs) owing to their ability to modulate the endocrine system. The detection of EDCs in the environment, biota and humans is of concern due to their potential to interfere with the physiology of living organisms (see Oehlmann *et al.* 2009).

In general, EDCs may disrupt the endocrine system by competing with endogenous steroid hormone binding to receptors and hormone transport proteins or by altering the metabolism or synthesis of endogenous hormones, eventually influencing recruitment of transcription factors and altering gene expression in cells (Wetherill *et al.* 2007). This is of particular concern for the developing organism, as it is sensitive to changes in the hormonal milieu, or drug or chemical exposure, which can result in organizational changes that are permanent (Guillette *et al.* 1995). This is in contrast to the situation in adults where changes in steroid hormones often lead to activational changes that are transient. Epigenetic changes have been seen following early developmental exposure to EDCs in animal studies, characterized by modified methylation patterns of genes leading to altered gene expression and phenotypic changes (Skinner & Anway 2007).

Human exposures to EDCs during this particularly vulnerable developmental time frame have been documented in a number of studies showing contamination of human breast milk (Norén & Meironyté 2000; Sun *et al.* 2004; Main *et al.* 2006), foetal liver (Schecter *et al.* 2007), amniotic fluid (Ikezuki *et al.* 2002) and cord blood (Schönfelder *et al.* 2002; Guvenius *et al.* 2003). Data from animal and human studies suggest

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that EDCs may play a role in the development of cancer (Takashima *et al.* 2008), the reported decline in human sperm count (Mocarelli *et al.* 2008), temporal increases in the frequency of developmental abnormalities of the male reproductive tract (Sharpe & Skakkebaek 2008) and the trend towards precocious puberty in human females (Schoeters *et al.* 2008).

Phthalates function as plasticizers to give flexibility to high-molecular-weight polymers and are found in soft plastic products (the addition of phthalates makes brittle polyvinyl chloride (PVC) soft). In addition, phthalates are also used as chemical additives in gel capsules, cosmetics and other personal-care products. Bisphenol A is widely used in the production of epoxy resins, polycarbonate plastics and brominated flame retardants (BFRs). BPA is the monomer (as opposed to an additive) used for production of polycarbonate plastic intended for food and beverage contact and many other products; it is also used to make resins that line metal food and beverage cans. TBBPA and PBDEs make up approximately 59 and 33 per cent of the world market of BFRs (Law *et al.* 2006), respectively, and are incorporated in a wide variety of materials including plastics intended for electronics and appliances as well as fabrics.

This review will present some aspects of human exposure to these compounds and investigations describing effects in animal models. The potential for human health effects based on evidence derived from experimental studies in animals will be discussed for each of these chemicals.

2. PHTHALATES

(a) *Human exposure*

Phthalate esters have recently attracted the special attention of the scientific community, regulatory agencies and the general public as a consequence of their high production volume, widespread use as plasticizers and chemical additives and possible endocrine-related effects (Mylchreest *et al.* 1999). Phthalates can easily leach out of products to contaminate the external environment because they are not chemically bound to the plastic matrix or to other chemicals in formulations. Di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used phthalate plasticizer for PVC (Matsumoto *et al.* 2002), but other compounds such as di-butyl phthalate (DBP), used as a fixative in cosmetics and other formulations, are also a cause of concern. Recent biomonitoring studies in the USA and Europe have detected relatively high levels of monoester metabolites of phthalates in the urine of the general population (Koch *et al.* 2004, 2006; Silva *et al.* 2004). In a recent study (Koch *et al.* 2006), the estimated median and 95th percentile of daily DEHP intake for a German population ($n = 85$) was 5.6 and 21 μg (kg bw^{-1}) d^{-1}, respectively. For children aged 3–14 years, these values were significantly higher—7.7 and 25 μg (kg bw^{-1}) d^{-1} ($n = 254$). Other studies have confirmed these data (reviewed by Heudorf *et al.* 2007). In addition, critically ill patients and neonates hospitalized in intensive care units may be exposed to significantly higher doses of phthalates that migrate from medical devices such as blood bags, catheters and nasogastric and intravenous tubes (Koch *et al.* 2006). Considering that developing organisms are particularly vulnerable to the effects induced by phthalates, newborns undergoing medical treatment in intensive care units may be at increased risk when compared with the general population. In a pilot study conducted by Koch *et al.* (2006), the daily DEHP intake of 45 neonates who were treated with various medical procedures was calculated. The median and 95th percentile was 0.042 and 1.780 mg DEHP (kg bw^{-1}) d^{-1}, respectively, and the maximum calculated intake was 2.3 mg DEHP (kg bw^{-1}) d^{-1}.

(b) *Experimental studies*

Although phthalates display low general toxicity, exposure to certain compounds is associated with disruption of endocrine and reproductive functions in experimental animals. The male reproductive tract seems to be particularly sensitive to phthalate exposure. Treatment of adult male rats with high doses of certain phthalates (e.g. 2000 mg DEHP kg^{-1} d^{-1}) results in rapid and severe changes in the testis (Gray & Gangolli 1986; Dostal *et al.* 1988). The observed alterations in spermatogenesis are thought to result from dysfunction in Sertoli cells, which cannot adequately provide physical and metabolic support to germ cells (Gray & Gangolli 1986). There is also experimental evidence showing that phthalates can target the Leydig cells and induce multiple hormonal disturbances (Akingbemi *et al.* 2004; Lin *et al.* 2008). However, most reproductive effects are not exerted by phthalate diesters themselves but rather by their active primary monoester metabolites formed in the liver, which are considered the proximate toxicants (Gray & Gangolli 1986). Recent evidence suggests that phthalates can also induce adverse responses in females following pre- and post-natal exposure (Grande *et al.* 2006, 2007; Gray *et al.* 2006).

Although most reproductive effects have been described in rats, phthalates can induce testicular injury in several other species including mice (Lamb *et al.* 1987), guinea pigs (Gray *et al.* 1982) and ferrets (Lake *et al.* 1976). However, some species, such as hamsters and non-human primates, seem to be less sensitive than rats (Gray *et al.* 1982; Foster *et al.* 1983; Kurata *et al.* 1998), and part of this variability may be attributed to differences in phthalate bioavailability (Foster *et al.* 1983; Ito *et al.* 2005). Accordingly, it has been argued that humans and non-human primates are less susceptible to the effects of phthalates, owing to the lower conversion of parent compounds into active monoester metabolites (Mckee *et al.* 2004). In a study by Kurata *et al.* (1998), no changes in testis weight or histopathology were observed in adult marmosets administered orally at 100, 500 or 2500 mg DEHP kg^{-1}d^{-1} for 13 weeks. More recently, the absence of testicular effects was also reported in young adult cynomolgus monkeys and juvenile marmosets exposed to high DEHP doses (500–2500 mg kg^{-1} d^{-1}) (Pugh *et al.* 2000; Tomonari *et al.* 2006). However, non-human primates have not been extensively evaluated during foetal and neonatal periods, which represent developmental windows especially susceptible to exogenous insults. A recent

work by Hallmark *et al.* (2007) indicates that neonatal marmosets treated orally with 500 mg kg^{-1} d^{-1} DBP respond similarly to rats in relation to changes in testosterone production and Leydig cell alterations, although marmosets seem to be able to reverse the suppression of testosterone production more efficiently than rats. Due to the lack of data on *in utero* and early post-natal exposures, it is not possible to draw conclusions regarding possible developmental effects in non-human primates.

In fact, the main concern involving phthalates is related to the effects induced during pre- and early post-natal development. Recent animal toxicity studies indicate that exposure to certain phthalates results in severe disorders in the developing male reproductive system, including defects in the external genitalia, undescended testes and testicular lesions. Reproductive toxicity induced during the perinatal period was reported for DEHP (Gray *et al.* 2000; Andrade *et al.* 2006*a*), DBP (Mylchreest *et al.* 1999), butyl benzyl phthalate (Gray *et al.* 2000; Nagao *et al.* 2000) and, to a lesser extent, for diisononyl phthalate (Gray *et al.* 2000). Male rat offspring exposed *in utero* or both *in utero* and during lactation to high phthalate doses (e.g. 500 mg DBP $kg^{-1}d^{-1}$) display reproductive tract abnormalities compatible with disruption of androgen-dependent development and impaired testicular function (Mylchreest *et al.* 1999; Gray *et al.* 2000; Andrade *et al.* 2006*a*). The phenotypic alterations manifested in male offspring include cryptorchidism, hypospadias, atrophy or agenesis of sex accessory organs, testicular lesions (e.g. small fluid-filled testes), reduced daily sperm production, delayed preputial separation, permanent retention of nipples and decreased (feminized) anogenital distance.

Unlike other anti-androgens, which act by binding to the androgen receptor and thus inhibit its ability to respond to androgens, phthalates disrupt the development of androgen-dependent structures mainly by inhibiting foetal testicular testosterone biosynthesis (Parks *et al.* 2000; Wilson *et al.* 2004; Howdeshell *et al.* 2008). This effect is mediated by changes in gene expression of enzymes and proteins involved in testosterone production by foetal Leydig cells, including the steroidogenic acute regulatory (StAR) protein, which participates in the transport of cholesterol to the inner mitochondrial membrane, the step in the steroidogenic pathway that is considered to be rate-limiting (Shultz *et al.* 2001; Lehmann *et al.* 2004). Recently, the expression of another product of the foetal Leydig cell, insulin-like factor 3 (Insl3), has been shown to be reduced in phthalate-exposed animals (Wilson *et al.* 2004). Such an effect might explain the incidence of retention of testes in the abdomen (cryptorchidism) following phthalate exposure, as Insl3 is involved in the initial stages of testicular descent into the scrotum (Wilson *et al.* 2004; Foster 2006).

Testicular histopathology resulting from phthalate exposure is seen early in the foetal testis with the presence of dysgenetic areas characterized by malformed seminiferous cords containing multinucleated gonocytes and aggregates of Leydig cells (Barlow & Foster 2003; Fisher *et al.* 2003). In adult offspring, affected testes display reduced germ cell differentiation, Sertoli cell-only (SCO) tubules, Leydig cell aggregates and multinucleated giant germ cells (Gray *et al.* 2000; Fisher *et al.* 2003; Andrade *et al.* 2006*b*). Reductions in Sertoli cell number and/or proliferation have also been reported in neonatal rats treated with phthalates (Dostal *et al.* 1988; Li *et al.* 2000). However, this appears to be a transient effect, as no changes in the number of Sertoli cells are observed later in life (Dostal *et al.* 1988; Andrade *et al.* 2006*b*).

In both adult and developing males, disturbance of Leydig and Sertoli cell functions constitutes integral effects of phthalates. In addition, gene profiling data obtained by microarray analysis indicate that phthalates affect similar genetic targets in pre-pubertal and foetal rat testes (Lahousse *et al.* 2006). However, hormonal and local cell signalling perturbations during early development may irreversibly alter reproductive and endocrine functions in a manner that may not be predicted from post-natal exposure. In addition, although several target genes involved in the development and function of foetal Leydig and Sertoli cells have been identified so far (Shultz *et al.* 2001; Lehmann *et al.* 2004), the mechanisms by which phthalates alter the expression of these genes are currently unknown.

(c) *Relevance of phthalate effects for humans*

Interestingly, the spectrum of effects obtained following perinatal exposure of male rats to phthalates has remarkable similarities with the human testicular dysgenesis syndrome (TDS). According to Skakkebaek *et al.* (2001), the human TDS is characterized by low sperm counts, cryptorchidism, hypospadias and testicular cancer, and the clinical expression of these symptoms may vary with the severity of the syndrome. Accordingly, less severe manifestations would result in impaired spermatogenesis while other symptoms such as testicular cancer may be present in more severely affected individuals (Skakkebaek *et al.* 2001). Similar to the human TDS, the effects induced by phthalates in rats constitute a continuum of response with the most severe manifestations and highest incidence of reproductive tract malformations observed at high doses (Foster 2006). *In utero* exposure of rats to active phthalates such as DEHP and DBP has been suggested as a useful animal model for human TDS, as in both humans and rodents disturbance of foetal Leydig and Sertoli cell functions plays a major role in induction of TDS-like symptoms (Fisher *et al.* 2003).

However, the main question involving phthalates is whether the level of human exposure is sufficient to adversely impact male and/or female reproductive health. A study by Swan *et al.* (2005) reported an association between phthalate exposure and reduced anogenital distance in human infants, an effect that is also observed in rats. However, the range of doses typically used in animal studies is three to four orders of magnitude greater than the estimated daily exposure of humans. Only recently have researchers begun to investigate and report on possible biological changes at doses within the range of median human phthalate exposure (Lehmann *et al.* 2004; Andrade *et al.* 2006*c*; Lin *et al.* 2008).

Some studies indicate that treatment of rat dams with active phthalates may result in non-monotonic (biphasic) dose–responses for the activity/expression of enzymes and proteins involved in the biosynthesis of steroid hormones in the offspring. Lehmann *et al.* (2004) studied alterations in genes coding for steroidogenic enzymes in the foetal testis of rats exposed to DBP. In this study, the authors reported reductions in several genes at doses that approach maximum human exposure levels. In a recent study with DEHP, Andrade *et al.* (2006*c*) have shown a striking biphasic dose–response for aromatase enzyme activity in the brain of neonatal males, with low-dose inhibition and high-dose stimulation. Since aromatase is a key enzyme in the biosynthesis of oestrogens, phthalate exposure might be associated with disturbances of the normal balance between androgens and oestrogens.

In addition to studies using low phthalate doses, recent studies have been conducted in order to evaluate the possibility of cumulative effects, since humans are exposed to multiple phthalates that are known to affect male reproductive development in rats. Howdeshell *et al.* (2007, 2008) demonstrated that different phthalates can act in a cumulative, dose-additive manner to reduce the testicular testosterone production by the rat foetal testis. Rider *et al.* (2008) reported dose-additive responses on reproductive malformations and androgen-dependent organ weights with developmental exposure to a seven-chemical anti-androgenic mixture that included some active phthalates. Coadministration of no-effect doses of DEHP and DBP reduces testicular testosterone levels and results in misshapen seminiferous cords and gonocyte multinucleation in the rat foetal testis (Martino-Andrade *et al.* 2008).

(d) *Conclusions*

Overall, the resemblance of phthalate effects in rats to human reproductive disorders has raised concerns over a possible link between phthalate exposure and human disease, even though most reproductive tract abnormalities in animal studies have been reported at dosages that probably result in internal dose concentrations that are well above those observed in humans. However, recent evidence for effects at lower doses as well as the results of combined toxicity studies and some epidemiological data on possible associations between phthalate exposure and reproductive effects in humans have provided further evidence for concern. The investigation of phthalate effects in non-human primates during pre- and early post-natal periods, the possibility of cumulative effects at low doses and a better understanding of the mode of action of phthalates and its relevance to humans are critical issues that should be addressed in future experimental studies.

3. BISPHENOL A

(a) *Sources and amounts of human BPA exposure*

Bisphenol A was reported to be a synthetic oestrogen (relative potency was not assessed) in 1936 (Dodds & Lawson 1936), 2 years before the oestrogenic activity of the structurally and functionally related drug diethylstilbestrol (DES) was described (Dodds *et al.* 1938). In the 1950s, polymer chemists discovered that BPA molecules could be polymerized to make polycarbonate plastic. Bisphenol A is also the base compound used in the manufacture of the resin lining of food and beverage cans in the USA and many other countries, although the Japanese can industry changed the formulation of the plastic lining of cans in the late-1990s. This voluntary action was reported to be associated with a loss of the previous correlation between use of canned drinks and urine levels of BPA in Japanese students as well as over a 50 per cent decrease in BPA levels (Matsumoto *et al.* 2003). These latter findings suggest that use of BPA-based resins to line cans contributes significantly to the human body burden of BPA. Food contact items (can lining, food packaging and food and beverage containers) are thought to be the major contributors to the median and mean values of approximately $2-4\ \mathrm{ng\ ml^{-1}}$ of unconjugated BPA detected in adult and foetal serum (Vandenberg *et al.* 2007). Pure water was poured into food cans that had contained different products and a polycarbonate food storage container. The products were heated to 100°C for 24 h, and BPA was analysed by HPLC with CoulArraay detection. The data presented in figure 1 reveal that all products leached detectable levels of BPA, although there were differences in the amount of leaching from different manufacturers' products. As expected, the cans that had contained the acidic tomato sauce resulted in the highest BPA values, since acid accelerates hydrolysis of the ester bond linking BPA molecules in polycarbonate and resins.

Another common use for BPA is as the monomer in dental sealants and composites used for fillings, from which BPA leaches in variable amounts and for different lengths of time depending on the product (Joskow *et al.* 2006). BPA is also an additive (referred to as a plasticizer) in PVC plastic products. For example, BPA has been reported to be present in PVC products such as stretch film (Lopez-Cervantes & Paseiro-Losada 2003). It is also used in printer ink and to coat paper used for receipts (referred to as 'carbonless paper'). It is thus a major contaminant in recycled paper products (Vandenberg *et al.* 2007). Polycarbonate food storage and beverage containers (the hard, clear containers, which may be tinted in the case of sport water bottles or baby bottles) cause concern regarding their potential to leach BPA because they are re-usable (Nerin *et al.* 2003), and repeated use leads to an increase in leaching (Brede *et al.* 2003). Many of these containers are marketed for use in the microwave, despite the fact that heating is known to increase BPA leaching levels. It is a basic characteristic of the ester bond linking BPA molecules together in polycarbonate plastic and resins that the rate of breaking of the bond by hydrolysis increases with heat, releasing free BPA (Bae *et al.* 2002), and an increase in leaching also occurs as a result of either an increase or decrease in pH (Welshons *et al.* 2006; Vandenberg *et al.* 2007). When food or liquids (such as beer) are placed into a can, they are heated to a high temperature for sterilization. The consequence is that food and beverages in cans have variable levels of BPA based on whether the contents are lipophilic

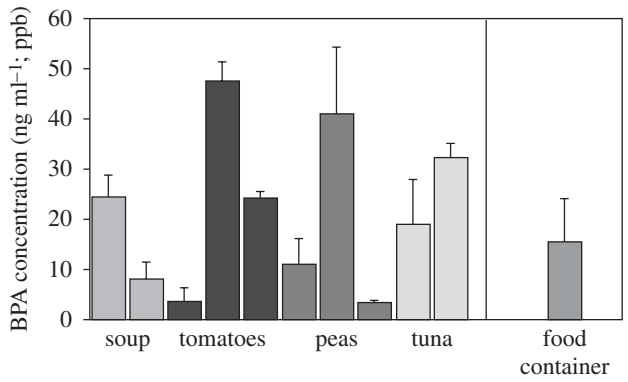

Figure 1. Bisphenol A (BPA) concentration in water placed into food cans that had contained different products. Two or three different brands of each product were examined. The cans were emptied, cleaned and rinsed with water that did not contain detectable levels of BPA. The 10 cans and a single new reusable 'microwave safe' polycarbonate food container were filled with HPLC-grade water and heated to 100°C for 24 h. Bisphenol A was extracted and analysed by HPLC with CoulArray detection (limit of detection was 0.01 ppb). Negative controls in glass bottles gave undetectable levels of BPA. Spiked samples in glass containers gave >95 per cent recovery. Significant leaching of BPA occurred from each type of product tested.

and/or acidic or alkaline (figure 1), all of which increase leaching (see the Environmental Working Group website for additional data on leaching of BPA from cans, at www.EWG.org).

A critical issue regarding routes of exposure to BPA was revealed by the Canadian Ministry of the Environment, which determined that BPA poses a threat to aquatic wildlife at current levels at which it is found in aquatic ecosystems (Canada 2008). This government report and other reviews (Vandenberg *et al.* 2007) identify that a number of studies show that BPA is leaching out of products being thrown into landfills and getting into ground water, contaminating rivers, streams and drinking water. While in an aerobic environment, BPA has a half-life of days, has a greater density than water and thus ends up in the sediment. In an anaerobic environment, BPA does not degrade. The Canadian Government is thus taking a regulatory approach to this problem that involves not only trying to limit the use of BPA in food and beverage containers, but also involves trying to deal with the problem of disposal of BPA-containing plastic that cannot be recycled to produce new BPA-based products.

The US Centers for Disease Control and Prevention (CDC) has measured BPA in the urine of people in the USA as part of the last national health survey (Calafat *et al.* 2008). The CDC reported that 93 per cent of people had detectable levels of BPA in their urine. Interestingly, the median and mean levels of unconjugated (parent) BPA reported in blood, as well as the lower and upper range reported for women and their foetuses at the time of parturition in Germany (Schönfelder *et al.* 2002), were virtually identical to values reported for total BPA in urine by the CDC. The findings by Schönfelder *et al.* are consistent with reports of blood levels of unconjugated BPA in people from other countries such as Japan (Vandenberg *et al.* 2007). In this review of the published literature, the authors determined that blood levels in humans identified by numerous analytical methods consistently showed approximately 10-fold higher median or mean levels of unconjugated BPA than average blood levels found throughout a 24 h period after laboratory rats were administered a BPA dose equal to the tolerable daily intake (TDI). The TDI corresponds to the amount of a chemical a person can be exposed to on a daily basis over an extended period of time (usually a lifetime) without suffering deleterious effects; this is also referred to by US regulatory agencies as the 'reference dose' and 'acceptable daily intake dose'.

Currently, a dose of 50 $\mu g\,kg^{-1}\,d^{-1}$ is considered 'safe' for daily human consumption by the US Environmental Protection Agency (EPA) (IRIS 1988) and Food and Drug Administration (FDA). Findings reported by Vandenberg *et al.* (2007) led to a consensus conclusion by 38 scientists who attended a US National Institutes of Health (NIH) sponsored conference on BPA (vom Saal *et al.* 2007) that current levels of human exposure to BPA already exceed the presumed safe daily exposure dose. The data presented in the review indicate that: (i) BPA is detected at the nanogram per millilitre (ppb) levels; (ii) unconjugated (bioactive) BPA is found in blood and (iii) the same levels are found in its conjugated (glucuronidated or sulphated) form in urine (Vandenberg *et al.* 2007). This is in sharp contrast to a review that concluded that BPA is rapidly and completely cleared from the blood of adults following oral absorption (Willhite *et al.* 2008). The conclusion that all ingested BPA is immediately metabolized (conjugated) to biologically inactive metabolites is thus not consistent with the published literature. The 'back calculation' approach of estimating human exposure to BPA based on the assumption that all orally absorbed BPA is immediately cleared from blood into urine is an invalid approach if the chemical is not immediately and completely metabolized (Barr *et al.* 2005).

BPA is one of the highest production volume chemicals in commerce, with over 6 billion pounds produced in 2003 (Burridge 2003), and a significant increase in production volume was expected at that time. In order to accurately estimate human exposure to BPA, we would need to know what products it is used in, since without this knowledge we have no way to determine the potential for different routes of exposure. This is currently not possible as identification of the chemicals used in products, such as baby toys, food and beverage containers or paper products, is not required. The latter is interesting in that printer ink contains BPA, and the 'carbonless paper' used to provide receipts for purchases is coated with BPA (the BPA reacts with dye in the paper in response to pressure or heat). Newspapers and carbonless paper could be significant sources of transdermal BPA exposure, and all recycled paper has BPA in it from these sources (Vandenberg *et al.* 2007). Dermal absorption of BPA from these potential sources of exposure has not been investigated.

(b) *Adverse health effects of BPA in laboratory animals*

There were three reports released in 2007 and 2008 in North America: two from the USA and one from Canada (discussed subsequently). The first was released as a consensus statement from an NIH-sponsored conference on BPA (vom Saal *et al.* 2007). This document was co-authored by 38 scientists who are experts in the field of endocrine disruption, and the majority of scientists at this conference had conducted research on BPA. The consensus document was a summary of the findings in five accompanying review articles that covered the entire published literature relating to the health effects of BPA as of the end of 2006 (Crain *et al.* 2007; Keri *et al.* 2007; Richter *et al.* 2007*a*,*b*; Vandenberg *et al.* 2007; Wetherill *et al.* 2007).

(i) *Adult exposure, experimental findings and relevance for human health*

There is extensive published literature showing the effects of acute exposure to very low doses of BPA in adult experimental animals: rats, mice and various aquatic species (Richter *et al.* 2007*a*,*b*). However, an issue that has generated much confusion is the rate at which BPA is metabolized. As described earlier, if one makes the assumption that virtually all BPA exposure is via an oral route and that virtually all BPA is immediately conjugated in the liver after absorption from the gut (transported from the gut to the liver via the direct hepatic portal vessels), then of course there would be no concern about BPA posing a threat to adults. However, this assumption is not consistent with the published biomonitoring literature regarding levels of unconjugated BPA in human blood (Vandenberg *et al.* 2007). Importantly, no rodent or human experiment has been conducted that involves chronic exposure, although the human biomonitoring data suggest virtual continuous exposure (Calafat *et al.* 2008). This is a major gap that needs to be filled.

The issue of route of exposure to BPA has generated significant controversy, since the default assumption is that oral exposure accounts for most (or virtually all) BPA exposure in humans. It is well known that exposure to BPA via injection, which does not result in the first-pass liver metabolism (glucuronidation/sulphation) that would occur following oral exposure, results in about a 10–20-fold higher amount of BPA in the blood relative to levels following oral administration (Vandenberg *et al.* 2007). However, if BPA is injected into rodents at doses of BPA that are thousands of times lower than the lowest adverse effect level (LOAEL) of 50 mg kg^{-1} d^{-1} that was used to establish the safe dose of 50 μg kg^{-1} d^{-1}, it is logical to assume that findings from studies that used injection as the route of administration are also relevant to assessing the potential health hazards posed by BPA, while recognizing that a correction for differences in pharmacokinetics based on route of administration is required. Just a few examples of effects of low doses of BPA in adult rodents are a significant stimulation of insulin secretion followed by insulin resistance in mice (Ropero *et al.* 2008), a significant decrease in daily sperm production in rats (Sakaue *et al.* 2001), a decrease in maternal behaviour in mice (Palanza *et al.* 2002) and disruption of hippocampal synapses, leading to the appearance of a brain typical of that seen in senility in both rats and monkeys (MacLusky *et al.* 2005; Leranth *et al.* 2007, 2008).

Related to the fact that type 2 diabetes is increasing in many regions of the world is the finding that exposure of adult mice to a low oral dose of BPA (10 μg kg^{-1} d^{-1}) resulted in stimulation of insulin secretion that was mediated by oestrogen receptor (ER) alpha, occurring as a rapid response mediated by the extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathway; and that the prolonged hypersecretion of insulin was followed by insulin resistance and postprandial hyperinsulinaemia (Alonso-Magdalena *et al.* 2005, 2008; Ropero *et al.* 2008). The fact that these very-low-dose studies of BPA are confirmed in cell culture studies that reveal molecular pathways that mediate effects of BPA in the low parts per trillion range has been reviewed (Wetherill *et al.* 2007). A consensus conclusion from the NIH-sponsored meeting on BPA was that the more recently discovered rapid-response pathway (as opposed to the nuclear ERs acting as ligand-activated transcription factors) may mediate many, but not all, of the low-dose effects of BPA (vom Saal *et al.* 2007).

The prediction from mechanistic studies in mice concerning the molecular pathways by which very low doses of BPA stimulate insulin production and secretion, which is then followed by insulin resistance (Ropero *et al.* 2008), leads to the fact that BPA may be related to elevated blood insulin and glucose, as well as insulin resistance. In addition, Hugo *et al.* (2008) reported that human adipocytes removed from different regions showed a marked suppression of the critical regulatory cytokine adiponectin, with the maximum response occurring at 1 nM (0.23 ppb), directly in the range of human exposure to BPA. A decrease in adiponectin is related to an increased risk for type 2 diabetes and cardiovascular disease and heart attack (Beltowski *et al.* 2008). It is thus of considerable interest that Lang *et al.* (2008) reported that in an analysis of data from 1455 people examined for BPA levels in urine as part of the US National Health and Nutrition Examination Survey (NHANES) conducted in 2003–2004, they found a significant relationship between urine levels of BPA and cardiovascular disease, type 2 diabetes and abnormalities in liver enzymes. This report, suggesting links between BPA and some of the most significant and economically burdensome human diseases, is based on a cross-sectional study and therefore cannot establish causality, but the fact that these findings are related to other studies that identify plausible mechanisms by which BPA at current levels of human exposure could result in these diseases greatly strengthens the importance of the findings (vom Saal & Myers 2008).

(ii) *Developmental exposure, experimental findings and relevance for human health*

The greatest concern regarding exposure to BPA is during development: foetuses, neonates, infants,

children and adolescents. The laboratory animal research on BPA is unique in that there are now hundreds of studies that have examined doses of BPA within the range of human exposure rather than the more typical approach in regulatory toxicology of only testing a few doses that are typically thousands of times higher than human exposure levels. The surprise associated with the first 'low dose' publication on the effects of BPA in laboratory mice in 1997 (Nagel *et al.* 1997) was that effects on the reproductive system in male offspring were found at a daily oral dose to pregnant mice that was 25 000 times lower than had ever been examined, and 25 times below the still current safe daily exposure dose according to the US FDA and US EPA, as well as the European Food Safety Authority (EFSA 2006). The lowest dose of BPA that had previously been examined was $50\ \mathrm{mg\ kg^{-1}\ d^{-1}}$ in some traditional very-high-dose toxicological studies conducted in the1980s. The $50\ \mathrm{mg\ kg^{-1}\ d^{-1}}$ was thus the LOAEL, and this dose was divided by 10 to 'estimate' the no observable adverse effect level (NOAEL) and divided by 1000 for the EPA to estimate that $50\ \mu\mathrm{g\ kg^{-1}d^{-1}}$ was safe for daily human exposure (IRIS 1988). Numerous reviews have been published discussing the assumptions used in estimating safe exposure levels for endocrine-disrupting chemicals such as BPA (vom Saal & Sheehan 1998; Markey *et al.* 2003; Welshons *et al.* 2003, 2006; vom Saal & Hughes 2005; Myers & vom Saal 2008).

One of the main concerns with the adverse effects reported in response to developmental exposure to very low doses of BPA (that produce blood levels in animals below those in humans) is that they all relate to disease trends in humans (table 1). For example, there is an obesity epidemic in many regions of the world, and developmental exposure to BPA increases body weight later in life (Howdeshell *et al.* 1999; Takai *et al.* 2000; Rubin *et al.* 2001). In addition, neonatal exposure to a low dose of DES ($1\ \mu\mathrm{g\ kg^{-1}\ d^{-1}}$) stimulated a subsequent increase in body weight and fat in CD-1 mice, while a dose 1000 times higher resulted in a significant decrease in body weight (Newbold *et al.* 2004).

Relevance for human health. The largest literature on the adverse effects of BPA exposure during development concerns adverse effects on brain structure, chemistry and behaviour (Richter *et al.* 2007*a*,*b*). One of the most interesting aspects of this literature is that there is a consistent finding of a loss of sex differences in brain structure, chemistry and behaviour owing to foetal/neonatal exposure to low doses of BPA. BPA thus appears to interfere with the normal processes that govern sexual differentiation, with brain changes reported in both males and females, depending on the outcome measured (Fujimoto *et al.* 2006; Rubin *et al.* 2006; Palanza *et al.* 2008). The implications at the population level for disruption of normal socio-sexual behaviours have not been extensively studied, although there are reports of changes in play behaviour (Dessi-Fulgheri *et al.* 2002) as well as other socio-sexual behaviours (Farabollini *et al.* 2002) that could impact population dynamics.

There are also numerous studies of the effects of low doses of BPA on the development of the female (Soto *et al.* 2008) and male reproductive organs in female rats and mice. Findings include chromosomal abnormalities in oocytes in females (Susiarjo *et al.* 2007), and long-term effects on accessory reproductive organs that are not observed until mid-life, such as uterine fibroids and para-ovarian cysts (Newbold *et al.* 2007). Other studies have shown that very low doses of BPA during pre-natal or neonatal development can result in permanent effects in male rats and mice. Low doses of BPA cause a decrease in daily sperm production and an increase in prostate size (vom Saal *et al.* 1998), an increase in prostatic androgen receptors (Gupta 2000; Richter *et al.* 2007*a*,*b*) and a progression from hyperplasia of prostate basal (stem) cells in the primary prostatic ducts during foetal life (Timms *et al.* 2005) to basal cell squamous metaplasia in adulthood (Ogura *et al.* 2007) and eventually to early stage prostate cancer (prostatic interepithelial neoplasia or PIN) in response to adult administration of testosterone and oestradiol (Ho *et al.* 2006).

Table 1. Prenatal–neonatal exposure of mice and rats to BPA at human exposure levels in relation to human health trends.

effects in mice and rats	human health trends
prostate hyperplasia and cancer	prostate cancer increase
mammary hyperplasia and cancer	breast cancer increase
abnormal urethra/ obstruction	hypospadias
sperm count decrease	sperm count decrease
early puberty in females	early sexual maturation
ovarian cysts/uterine fibroids	polycystic ovary syndrome/ uterine fibroids
abnormal oocyte chromosomes	miscarriage
body weight increase	obesity increase
insulin resistance	type 2 diabetes
hyperactivity/impaired learning	attention deficit hyperactivity disorder

(c) *Conclusions*

The required testing of chemicals for regulatory purposes is not aimed at understanding molecular mechanisms and focuses on effects occurring at high doses that are typically not relevant for human exposure scenarios. Over the last 10 years, there has been a dramatic shift in the approach of scientists with regard to investigating doses that are relevant for human exposure to study the effect of BPA in laboratory animals. This has led to a totally unique toxicological literature revealing extensive evidence that effects in laboratory animals are occurring at blood levels that are lower than those found in the average person in a developed country. This is clearly of great concern for possible impact on human health.

The assumptions used in chemical risk assessments, such as all dose–response curves are monotonic, and there is a threshold dose below which no effect

occurs at which the system is 'off', clearly do not apply to endogenous hormones, hormonally active drugs or hormonally active chemicals. How can there be a threshold at which no oestrogen responses occur for a chemical that is adding oestrogenic activity to a system that is already 'on' at zero dose of the exogenous chemical (Sheehan *et al.* 1999; Sheehan 2005)? Also, all hormones show responses that are non-monotonic, and non-monotonic dose–response curves have been reported in many BPA studies (Richter *et al.* 2007*a*,*b*; Alonso-Magdalena *et al.* 2008). The need to integrate concepts of endocrinology in the assumptions underlying chemical risk assessments will some day result in the development of a new system, and the data from findings with low doses of BPA will play an important role in this paradigm shift (Myers & vom Saal 2008).

4. BROMINATED FLAME RETARDANTS

(a) *Uses of TBBPA*

Brominated flame retardants function by increasing the time between ignition of a fire and flash over, which is the point when enough heat is generated to cause combustion of flammable materials. TBBPA is the classical halogenated flame retardant chemically bonded to epoxy and polycarbonate resins. It is present in printed circuit boards and casings used in personal computers, printers, fax machines and copiers. Dimethyl TBBPA is added to acrylonitrile–butadiene–styrene (ABS) resin and high impact polystyrene (HIP). This derivative is blended with the polymers, meaning that it exists free in the chemical matrix. ABS resins have a wide variety of applications including automotive parts, pipes and fittings, and domestic and office appliances. Polystyrene is used in packaging, electrical and electronic equipment enclosures for televisions, furniture and construction materials. Another derivative, bis (2-hydroxyethyl ether) TBBPA is used as a flame retardant for paper and textile adhesives and coatings (Alaee *et al.* 2003).

(b) *Exposure to TBBPA*

Products with both additive and chemically bonded forms of TBBPA have been shown to release TBBPA into the environment (Birnbaum & Staskal 2004), resulting in detection in sewage sludge (Oberg *et al.* 2002), soil, sediments, birds, fish (Morris *et al.* 2004) and air from different occupational settings (Sjödin *et al.* 2001). TBBPA serves as a source of environmental BPA as it has been shown to break down to BPA in marine sediments (European Union Risk Assessment Report 2005).

Although studies indicate high first-pass metabolism of TBBPA in rats and humans owing to rapid conjugation with glucuronic acid and elimination in the bile (Kuester *et al.* 2007), TBBPA has been detected in cow and human milk (Thomsen *et al.* 2002*a*; Antignac *et al.* 2008), human serum (e.g. Hayama *et al.* 2004), human adipose tissue (Johnson-Restrepo *et al.* 2008) and umbilical cord serum (Antignac *et al.* 2008). Evaluation of BFRs in archived human serum samples in Norway showed a temporal increase in concentrations of six PBDE congeners and TBBPA over the period 1977–1999. The concentrations for different age groups were relatively similar, except for the 0–4 year olds who had 1.6–3.5 times higher serum concentrations (Thomsen *et al.* 2002*b*).

(c) *Experimental studies on TBBPA*

There are only a few published studies regarding the toxicology of TBBPA. Information regarding the endocrine-disrupting potential of TBBPA mainly comes from *in vitro* studies and *in vivo* studies performed in quail, fish and tadpoles; however, two papers describing a study in the rodent model have been recently published.

(i) *Thyroid hormone effects*

An increase in thyronine (T3) in female rat offspring and a reduction in circulating total thyroxine (T4) were observed in both sexes in a reproductive study with exposure to TBBPA in food, beginning 70 or 14 days prior to mating of F0 males and females, respectively, and which continued throughout gestation and lactation up to 14 weeks of age at necropsy (Van der Ven *et al.* 2008). Thyroid hormone concentrations were similarly altered at 12 weeks of age in a subacute toxicity study (28 days of repeated dosing of TBBPA) with a significant decrease in T4 and increase in T3 in males. Parallel changes were observed in females; however, they were not statistically significant (Van der Ven *et al.* 2008). In the same reproductive experiment, appraisal of brainstem auditory evoked potentials (BAEP), a means to detect effects on hearing, indicated changes in hearing latency and hearing threshold similar to previous reports on developmental exposure to polychlorinated biphenyls (PCBs) (Lilienthal *et al.* 2008). It is plausible that the changes in thyroid hormone status mediated these effects as thyroid hormones play a crucial role in the development of auditory function, and the benchmark dose levels for changes in thyroid hormones (2.3–30.8 mg kg^{-1} d^{-1}) and BAEP (1–40 mg kg^{-1} d^{-1}) were in the same range (Van der Ven *et al.* 2008).

(ii) *Binding to transthyretin*

TBBPA has an even closer structural relationship to T4 than PCBs. *In vitro* competitive binding assays demonstrated that TBBPA binding to human transthyretin (TTR) is more potent than the natural ligand (Meerts *et al.* 2000; Hamers *et al.* 2006). It is theorized that these compounds may decrease serum T4 concentrations by displacing it from the carrier proteins, leading to increased clearance. This displacement could also make more T4 available for deiodination, leading to increased formation of T3 or reverse T3.

(iii) *Binding to thyroid hormone receptor and thyroid hormone activity*

During development, most tadpole tissues are influenced by thyroid hormones, making them suitable to evaluate potential disruption of thyroid homeostasis. An *in vivo* study simultaneously exposing *Rana rugosa* tadpoles to T3 and TBBPA found TBBPA

suppression of T3 induced tail shortening, indicating a thyroid hormone antagonist effect (Kitamura *et al.* 2005*a*). While only mild effects on larval development were observed, a study in *Xenopus laevis* tadpoles also suggested thyroid hormone antagonism by TBBPA (Jagnytsch *et al.* 2006). *In vitro*, TBBPA competes with T3 binding to a nuclear suspension from rat pituitary Mt/T/e-2 cells (Kitamura *et al.* 2002) and was shown to inhibit the activity of T3 in Chinese hamster ovary cells transfected with human TRα or TRß (Kitamura *et al.* 2005*a*).

(iv) *Sex steroid effects*

An increase in pituitary weight in male offspring was noted in the reproductive study (Van der Ven *et al.* 2008), which correlates with previously published *in vitro* findings from Kitamura *et al.* (2002, 2005*b*), showing that TBBPA increased proliferation of GH3 cells (rat pituitary cell line) and growth hormone production. Ghisari & Bonefeld-Jorgensen (2005) also observed that TBBPA stimulates GH3 cell growth, which was inhibited by the anti-oestrogen ICI 182 780, suggesting that this effect is ER-mediated.

In vitro studies indicate that the lower brominated forms of TBBPA have higher affinity with the ER than the higher brominated analogues (Samuelsen *et al.* 2001). In MCF-7 human breast cancer cells, TBBPA functioned as a partial ER agonist (Olsen *et al.* 2003) and demonstrated mixed agonist/antagonist activities in estrogen responsive element (ERE)-luciferase reporter gene assays. The oestrogenic activity was confirmed *in vivo* using the uterotrophic assay (Kitamura *et al.* 2005*b*).

Inhibition of oestradiol sulphation by TBBPA has been reported *in vitro* (Kester *et al.* 2002; Hamers *et al.* 2006), which suggests the potential for impaired elimination *in vivo* leading to increased biologically active oestradiol concentrations. Environmentally relevant TBBPA concentrations have been shown to decrease reproductive success in zebra fish (Kuiper *et al.* 2007) and inhibition of oestradiol metabolism in lake trout (Jurgella *et al.* 2006). In male Japanese quail, however, no oestrogenic effects after embryonic exposure to TBBPA were observed for reproductive behaviour, plasma testosterone and testicular morphology (Berg *et al.* 2001; Halldin *et al.* 2005).

(d) *Products with PBDEs*

Two (penta and octa formulations) of the three main commercial mixtures of PBDEs added to polymers for the manufacture of goods are no longer in production. The penta mixture was applied to polyurethane foams used in furniture, mattresses, carpet pads and automobile seats and styrene plastics used for electrical appliances and flame-retardant textiles. The octa-technical mixture was blended with ABS resins used in automobile electronics, home and office appliances and high-impact sport equipment and toys. The deca product is added to a variety of polymers, and examples of end products include fabric backings and housings for electronics (Frederiksen *et al.* 2008).

(e) *Exposure to PBDE*

The presence of PBDEs in breast milk, adipose tissue and serum has been confirmed in several studies (as reviewed in Frederiksen *et al.* 2009). An examination of a cohort of 4-year-old children revealed that breast-fed children have 6.5 times higher average body burden of total PBDEs than formula-fed children (Carrizo *et al.* 2007). Detection of PBDEs in liver tissue of human foetuses (Schecter *et al.* 2007) and in cord blood (e.g. Antignac *et al.* 2008) demonstrates that *in utero* exposure is taking place. On a pro-kilogram basis, studies have described higher levels of exposure to PBDEs for children than adults (as reviewed in Frederiksen *et al.* 2009).

(f) *Animal studies investigating developmental exposure to PBDEs*

There are more published studies regarding effects following *in vivo* exposure to PBDEs than for TBBPA. Animal studies have revealed the potential for endocrine disruption and effects on neurobehaviour and the reproductive system.

(i) *Thyroid hormone*

In rodent studies, perinatal or peripubertal exposure to PBDEs causes a reduction in T4 while effects on T3 and TSH are less consistent. Perinatal exposure to 1, 10 or 30 mg kg^{-1} d^{-1} DE-71 (commercial penta-mixture) (Zhou *et al.* 2002) or 18 mg $kg^{-1}d^{-1}$ (Ellis-Hutchings *et al.* 2006) did not alter T3 concentrations. However, a concomitant decrease in T3 and an increase in thyroid stimulating hormone (TSH) in males only were observed following peripubertal exposure to 30 or 60 mg kg^{-1} d^{-1} DE-71 (Stoker *et al.* 2004), and another study reported a decrease in T3 in female rats following exposure to 100 or 300 mg kg^{-1} d^{-1} DE-71 or 60 or 100 mg kg^{-1} d^{-1} DE-79 (octa-commercial mixture) on post-natal day (PND) 28–32 (Zhou *et al.* 2001). Administration of 300 µg kg^{-1} d^{-1} BDE-99 (2,2′,4,4′,5-pentabromodiphenyl ether) on gestation day (gd) 6 resulted in a transient decrease in T3 in male offspring on PND 1 with reductions in T4 occurring in both male and female offspring on PND 22 (Kuriyama *et al.* 2007).

(ii) *Effects on sex steroids and the reproductive system*

Following gestational exposure, *in vivo* oestrogen activity for BDE-99 has been indicated by increased expression of mRNA of oestrogen responsive genes in the uterus (Ceccatelli *et al.* 2006). Alterations in the sex hormone profile have been reported, including reductions in circulating oestradiol and testosterone in adult male offspring (Lilienthal *et al.* 2006) and lower circulating oestradiol concentrations without changes in whole ovarian aromatase activity in young female rat offspring exposed to 700 µg kg^{-1} of BDE-47 on gd 6 (Talsness *et al.* 2008). Possible impairment of androgen function following high-dose peripubertal exposure to DE-71 was indicated by postponed puberty and delayed growth of androgen-dependent tissues in male rats without an effect on circulating testosterone concentrations (Stoker *et al.* 2004). Additional *in vitro* studies by the same group suggest

that the observed effects may be because of binding of some of the congeners in the mixture acting as antagonists to the androgen receptor (Stoker *et al.* 2005).

Functional changes to the gonads have been observed following *in utero* exposure to PBDEs. Administration of an agent used to treat hyperthyroidism, 6-*n*-propyl-2-thiouracil (5 mg l^{-1} drinking water on gd 7–21), or 60 or 300 μg BDE-99 kg^{-1} on gd 6 (Kuriyama *et al.* 2007) resulted in a reduction in spermatid and sperm counts without an influence on circulating testosterone concentrations in adult F1 males (Kuriyama *et al.* 2005). Whether the impaired spermatogenesis is accompanied by changes in the testicular cell population based on DNA ploidy (flow cytometry analysis) was examined in isolated testicular cells from the other testis (12 per group) of the same animals. DNA staining was performed by suspending the cells in phosphate buffered saline with 100 μg ml^{-1} ribonuclease A and 50 μg ml^{-1} propidium iodide. Propidium iodide fluorescence was measured at 620 nm to differentiate between mature haploid cells (elongated spermatid), immature haploid cells (round/elongating spermatid) and diploid cells (spermatogonia, spermatocyte, Sertoli cells, Leydig cells). The number of Sertoli cells in the seminiferous epithelium is related to sperm production since each cell supports a finite number of germ cells (Russel *et al.* 1990). This cell number was also determined in histological sections from other F1 males (six per group) on PND 110. Sertoli cell nucleoli were counted in 25 round or nearly round seminiferous tubule cross-sections chosen at random, and 25 Sertoli cell nucleoli diameters were measured for each animal. These counts were corrected for section thickness and the smallest recognizable nucleolar profile (cap section) as described previously (Russel *et al.* 1990).

A statistically significant decrease in the ratio of haploid/diploid cells was observed in the PBDE-99 group (figure 2), which corroborates the reduced number of spermatid/sperm counts observed. We observed slight differences in the ratio of mature haploid (mostly elongated spermatid)/diploid cells in treated animals, and this effect was more pronounced when the ratio of immature haploid (round/elongating spermatid)/diploid cells was compared with controls. No change in the number of Sertoli cells/seminiferous tubule cross-section was observed among the groups (figure 2), indicating that the reduction in haploid cells is because of another cause and supporting the finding that the changes in these ratios are owing to reductions in the haploid populations. The observed impaired spermatogenesis in male offspring exposed to low-dose BDE-99 during development (Kuriyama *et al.* 2005) has been confirmed by flow cytometry analysis and is not mediated by reduced numbers of Sertoli cells. Alternatively, a hormonal-dependent mechanism can be proposed as sperm production is dependent on permissive actions of follicle stimulating hormone (FSH) and testosterone and, therefore, luteinizing hormone (LH). Although significant differences in testosterone and LH concentrations in serum from adult animals were not found, the possibility of hormonal changes during early development cannot be ruled out. Functional changes in the seminiferous epithelium leading to impairment of normal spermatogenesis in adult rat offspring can also be speculated.

In adult female offspring, ultrastructural changes in the ovary following low-dose gestational exposure to either PBDE-99 (Talsness *et al.* 2005) or BDE-47 (Talsness *et al.* 2008) and effects on folliculogenesis after administration of 1 or 10 mg kg^{-1} d^{-1} of BDE-99 during gestation (Lilienthal *et al.* 2006) were observed. Altered folliculogenesis evaluated around the time of puberty after low-dose exposure to BDE-47 during gestation has also been reported (Talsness *et al.* 2008). The changes in the latter study were characterized by a reduction in antral follicle numbers and circulating estradiol concentrations. Whether these changes are because of thyroid hormone disruption or alterations in gonadotropins and/or sex hormones is unclear.

The animal data thus far suggest that PBDEs may affect the reproductive system via alterations in thyroid hormone profile and/or sex steroid action. Aberrations in thyroid hormone status are known to adversely affect reproduction. In animal studies, thyroid hormone has been shown to play an integral role in testicular development (Cooke *et al.* 1992) and affect ovarian follicular maturation (Baldridge *et al.* 2004).

(iii) *Neurobehaviour*

The period of rapid brain growth represents a particularly vulnerable developmental window to insults and is characterized by a dramatic increase in the number of cells and myelination, cell migration, dendritic and axonal growth and the formation of neural connections. Exposure to PBDEs during the brain growth spurt of mice modified spontaneous motor behaviour and habituation to new surroundings (e.g. Viberg *et al.* 2006) and altered levels of proteins involved in brain maturation (Viberg *et al.* 2008). Other studies have found changes in spontaneous motor activity following early post-natal exposure to mice, as well as effects on one measure of the ontogeny of sensorimotor integration (Rice *et al.* 2007) and subtle differences in neuromotor development (Branchi *et al.* 2002; Gee & Moser 2008). In addition, examination of cognitive function revealed deficits as mice exhibited impaired learning and memory on the Morris water maze test following early post-natal PBDE exposure (Viberg *et al.* 2003).

Administration of BDE-99 to rats during gestation at a dose (300 ug kg^{-1} on gd 6) resulting in dam adipose tissue concentrations (Kuriyama *et al.* 2007) only slightly higher than those reported for this congener in humans (Johnson-Restrepo *et al.* 2005) led to hyperactivity in the rat offspring (Kuriyama *et al.* 2005).

(g) *Relevance of exposure to flame retardants for human health*

(i) *Reproduction*

It is known that diseases of the thyroid gland affect the reproductive capacity of women. In particular, hypothyroid women may exhibit anovulation, and hypothyroidism is associated with hyperprolactinaemia,

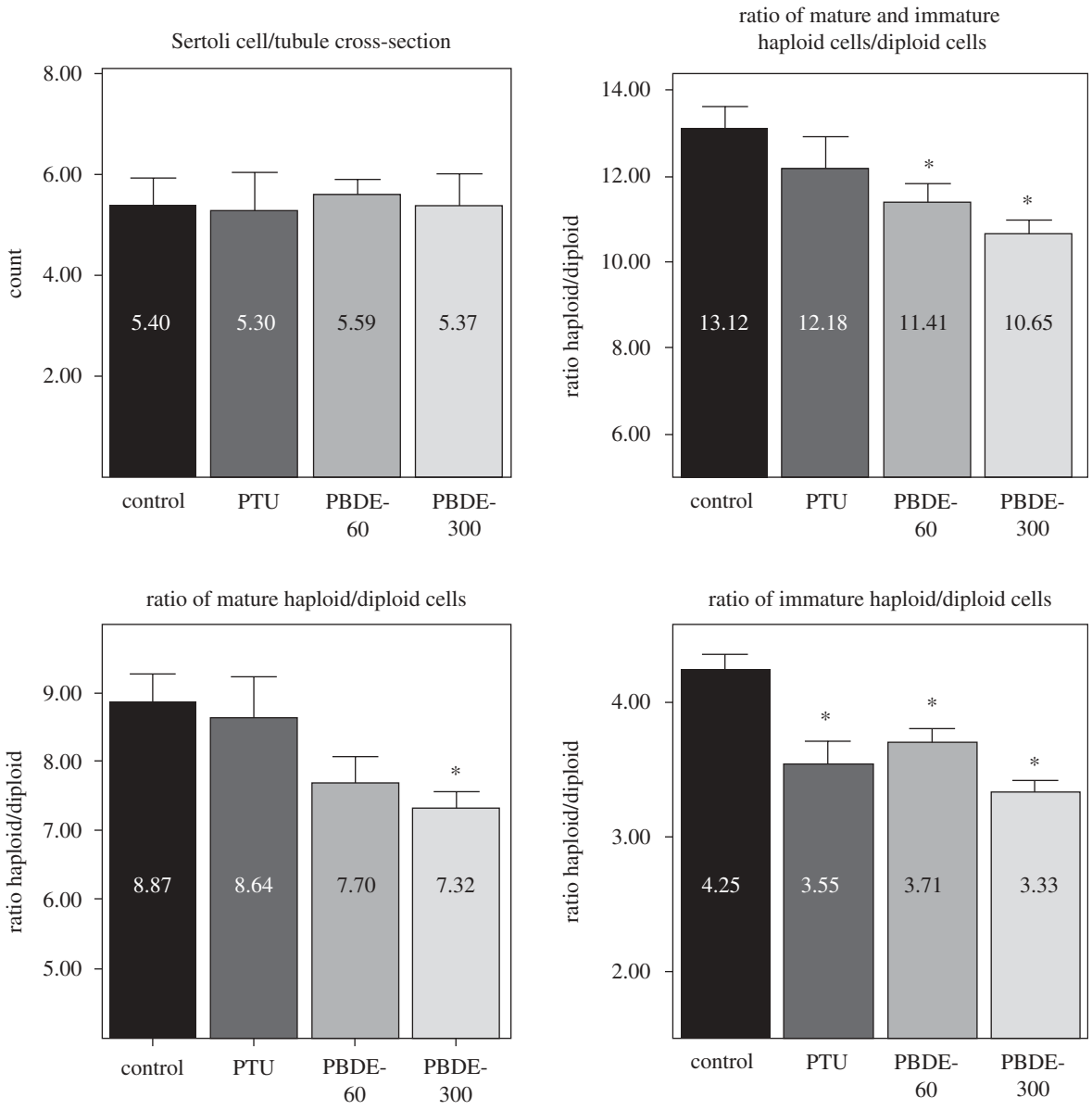

Figure 2. Number of Sertoli cells per tubule cross-section in adult male offspring (F1) on PND 110 and flow cytometry analysis of testicular cell population on the basis of their DNA ploidy in adult rats on PND 140. Gravid female rats were administered per gavage either vehicle (control) or 60 or 300 μg BDE-99 (kg bw)$^{-1}$ (PBDE-60 or PBDE-300) on gd 6. A fourth group received 5 mg 6-*n*-propyl-2-thiouracil (PTU) l^{-1} in drinking water from gd 7–21. ANOVA followed by Dunnett *t*-test, $^{*}p < 0.05$.

which inhibits the release of pituitary gonadotropin and gonadal steroids.

Although less is known about the role of thyroid hormones in the development of the reproductive system, it is believed that they are important for gonad development in both sexes (Cooke *et al.* 2004). Animal studies and *in vitro* studies demonstrate that TBBPA and PBDEs interfere with thyroid hormone action, and the function of both the ovary and the testis has been altered following *in utero* exposure to PBDEs. Thyroid hormones indirectly affect sperm production by regulating the number of Sertoli cells, which act as nurse cells for developing sperm. It should be noted, however, that the new data presented here indicate that the observed reduction in sperm count following gestational BDE-99 exposure was not associated with changes in Sertoli cell number. In the ovary, thyroid hormone receptors are present in granulosa cells, which produce steroids and provide growth factors interacting with the oocyte.

In addition, alterations in sex steroid concentrations or function and LH levels have been reported in animal studies for PBDEs, indicating the potential to adversely affect human reproduction. Two epidemiological studies indicate that exposure may be associated with adverse affects on the testis. Preliminary data from a pilot study performed in Japan suggest an inverse relationship between BDE-153 (2,2′4,4′,5,5′-hexabromodiphenyl ether) serum concentrations and sperm concentration in young Japanese males (Akutsu *et al.* 2008). A larger study relating information regarding *in utero* and early developmental exposure to PBDEs with cryptorchidism involved 86 Danish and Finnish newborn boy–mother pairs. Associations between PBDE contamination of human breast milk and cryptorchidism and

increased gonadotropin release to support normal testosterone production were observed (Main *et al.* 2007). Although there was no significant difference in the placental concentrations, the sum of PBDEs in breast milk was higher in cryptorchid boys than in controls and was positively correlated with infant serum LH concentration. The authors suggest that the lack of correlation between the PBDE concentrations in the placental and breast milk samples may be that the placenta represents the situation at delivery as one would find in a single blood sample and not the long-term exposure.

Although the exposure time frames are not comparable, an increase in LH was also reported for adult male rats exposed to 60 mg kg^{-1} d^{-1} of DE-71 for 3 days (Stoker *et al.* 2005). As indicated previously, Insl3 and androgen play roles in testicular descent. LH regulates testosterone production and plays a role in the differentiation of Leydig cells, which must reach a certain stage before production of Insl3 occurs (Sadeghian *et al.* 2005). The rate of this maturation process in rats has been reported to be influenced by T3 (Mendis-Handagama *et al.* 2007). Alterations in thyroid hormone profile or changes in the status of the hypothalamo–pituitary–testicular axis could influence testicular descent by impacting Insl3 formation.

(ii) *Neurodevelopment*

Thyroid hormone is crucial for growth and development and in particular, for neurodevelopment. Decreases in foetal and maternal thyroid hormone are known to impact neuropsychological development in humans, and impaired achievement on neuropsychological tests can occur even when maternal hypothyroidism is subclinical (Haddow *et al.* 1999). An evaluation of human thyroid hormone status and PBDE exposure was performed in China. TSH and serum concentrations of PBDEs were found to be significantly increased in subjects living close to an electronic waste site compared with those living 50 km away, suggesting hormone disruption (Yuan *et al.* 2008).

Aberrations in thyroid hormone homeostasis caused by PBDEs and TBBPA raise concerns regarding their potential to influence child neurodevelopment. Animal studies indicate changes in behaviour following exposure to PBDEs during a critical developmental window, which may be mediated through changes in thyroid hormones or direct neurotoxicity.

Data regarding neurotoxicants indicate that there is a continuum of toxic outcomes at low doses, i.e. chronic daily exposures, which do not induce overt clinical symptoms (Grandjean & Landrigan 2006). The authors suggest that neurodevelopmental disorders associated with exposure to known human neurotoxicants and untested chemicals have resulted in a veiled pandemic, incurring significant costs to society because of lowered productivity and reductions in intelligence (Grandjean & Landrigan 2006).

5. GENERAL CONCLUSIONS

Exposure of humans to pharmaceuticals is deliberate, with the intention of achieving a desired effect. Development and testing of medications involves a series of evaluations culminating in human clinical trials before marketing is approved. This is quite different from the situation with chemicals, whose presence in biota and humans is inadvertent. In the field of toxicology, information regarding potential human health effects is mainly derived from experimental studies and, when available, from epidemiological studies. Difficulties are not only encountered with extrapolation from animal models to humans, but epidemiological studies are also thwarted by drawbacks such as controlling for confounding factors. In particular, subjects are exposed to an assortment of chemicals on a daily basis and, often, lack of data regarding the extent of exposure at what may have been the critical time frame. One of the goals of toxicology is to identify effects in animal models with the aim to lower the risks of negatively impacting human health. Implicit in this task is that toxicological data, derived from animal studies indicating a potential for adverse effects, serve as a basis to limit exposure before effects appear or are confirmed in humans. The evidence from animal studies on single exposures to the chemicals discussed here suggests the potential for risk to human health. Moreover, data derived from co-exposure studies support the contention that the assortment of chemicals to which we are exposed on a daily basis increases the likelihood of health effects. The high prevalence of body burdens of these chemicals and simultaneous exposure to a number of substances, in conjunction with the fact that the highest concentrations have been demonstrated in the developing young, a sensitive subpopulation of society, indicate the need to decrease the exposure to these compounds.

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