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Online Enterprises • 245 Visitacion Ave, Brisbane, CA 94005
Phone: (415) 467-8779 • Fax 415-468-3144• e-mail: info@eeletter.com

Bisphenol A Concerns Survey: An E/E Letter Special Report

Introduction

The infrastructure behind the Internet revolution rode on a wave of compact disks made from Bisphenol-A, the main ingredient in polycarbonate plastic. BPA is also playing a significant role in new baby bottles, lighter windshields, metal can liners, and epoxy glues.

Yet at the same, a plethora of recent research has suggested that BPA may influence a wide range of health concerns including obesity, enlarged prostates, the effectiveness of prostate cancer medication, and changes in behavior. This survey represents an effort to highlight some of the concerns that have been presented about BPA, and their potential significance. Consequently more space is given to the concerns that have been raised in this survey, even though an even larger body of research has been conducted, which suggests there are no low-dose concerns around BPA.

Interest in the subject is keen on both sides of the debate, with a $2.2 billion (Greiner et al.2001)1 direct market at stake, and indirectly hundreds of billions when one considers the value of software and other products delivered using BPA.

Industry and governments continue to state that the weight of evidence does not appear to support cause of concern. Meanwhile advocacy groups are pointing to a growing body of studies suggesting that BPA is capable of causing health effects at levels to which humans are exposed. A large number of studies have shown effects in the test tube, and in live animals. While at least one study suggesting low dose effects have been repeated within the same lab, no in-vivo experiments suggesting low-dose effects have been repeated across labs.

Researchers and activists have suggested that this is due to funding priorities, since few institutions are ready to support research that is not looking for something new. Industry on the other hand suggests that this is an indication that low dose effects don't really exist. Meanwhile about 2 million metric tons are flowing into CDs, baby bottles, and other products every year.1 Another report estimates the market grew from 600,000 to 1.8 million tons between 1990 and 2000.2

Toxicological Controversy

As early as 1938, researchers reported that BPA was estrogenic, when given to animals at fairly high doses.3 BPA was mostly just a scientific curiosity until 1953, when Dr. Daniel Fox at General Electric and Dr. Hermann Schnell at Bayer AG discovered polycarbonate plastic independently. Over time it found its way into space helmets, car parts, computers, cell phones, glasses, and compact disks.

While the effects noted by Dodds et al were interesting from a scientific point of view, they only seemed to occur at concentrations orders of magnitude higher than what people were likely to be exposed to. A comprehensive analysis of BPA by Morrisey et al. (1987) set the lowest observed adverse effect level at 50,000 ppb of bodyweight/day.4

The toxicological concerns around BPA were more or less settled until 1997 when a team at

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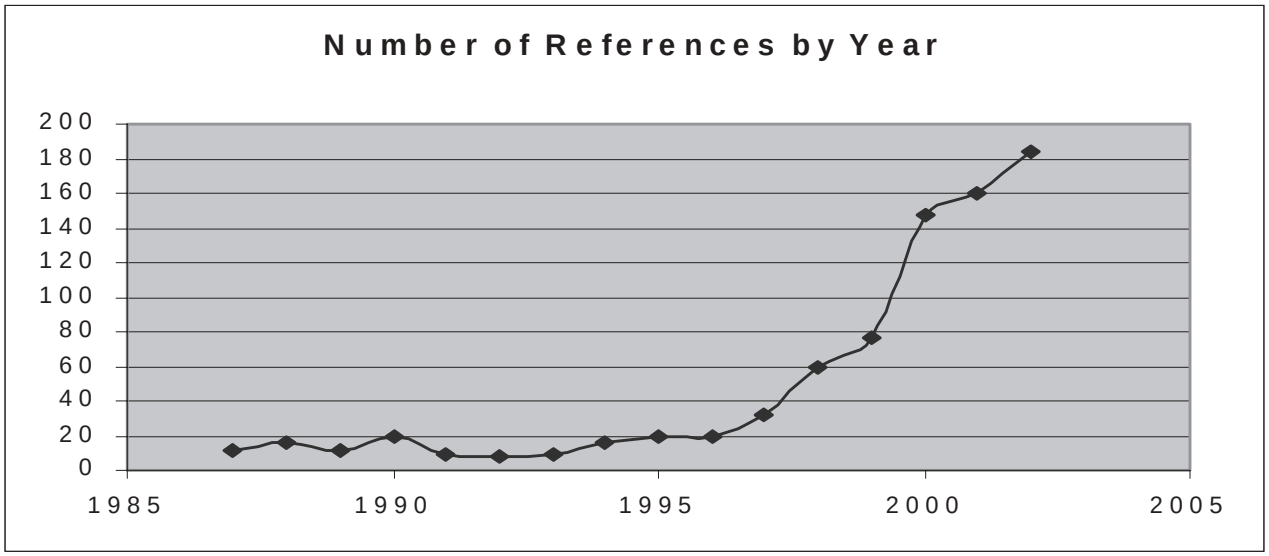

Table 1: BPA Reference Citations Per Year
A survey of the number of BPA papers on STN Caplus restricted to biology showed the number of papers on BPA almost doubled from 32 to 60 in 1998 and rose to 184 in 2002.

Frederick Vom Saal's lab reported on BPA effects at only 2 ppb.5 The study received plenty of criticism due to its small size (only seven animals analyzed) and the lack of repeatability. But at the same time it opened the gates for a plethora research in other labs.

Researchers like Dr. Makoto Ema, with the National Institute of Health Sciences Biological Safety Research Center Division of Risk Assessment in Japan believe these findings are still valid, noting, "There is not a definitive report that denied the findings described by vom Saal et al." But Ema also noted, "It is important to distinguish whether the findings reported by vom Saal et al. are toxicologically adverse or not."

Dr. Ibrahim Chahoud, a researcher with Freie Universitaet, Institute of Clinical Pharmacology and Toxicology in Berlin, said, "I believe the concerns regarding BPA are still valid. There are several positive and negative observational studies in the literature and until this is resolved, one needs to remain concerned. There may be a subpopulation, i.e. the developing organism, that is more sensitive to the effects of endocrine active compounds than an adult." He also noted, "The most important endpoints would include those which would help us to understand the mechanism of action of BPA in vivo."

Chiharu Tohyama with Environmental Health Sciences Division National Institute for Environmental Studies, and who led the Sakaue study, said, "I realize that quite a few papers have come out in the last 2 years, showing that low dose BPA affects laboratory animals in terms of some parameters, such as immunologic response, chromosomal change, sexual differentiation of the brain and sexual behavior. These experimental results strongly suggest that exposure to BPA may have some adverse effects in the offspring.

"On the other hand, BPA may not be adverse in the adult male animals in terms of fertility. We found that low dose BPA treatment to adult rats resulted in a clear-cut dose-dependent decrease in spermatogenesis in the adult animals in a dose-dependent manner (from .002 to 200,000 ug/kg b.w.), but we would speculate that daily sperm production levels were still high enough for fertility even at the highest dose of BPA used (200,000 ug/kg b.w.), although this speculation remains to be proven."

Tohyama noted that, "sexual differentiation of the brain, brain functions, immunologic functions, and chromosomal alterations should be carefully

studied with low-dose BPA exposure."

Toshihiro Takao with Kochi Medical School, in Nankoku, Japan, said, "The most important endpoints are the effects for human male reproduction."

Dr. Daniela Buckiova, with the Institute of Experimental Medicine Academy of Sciences in the Czech Republic said, "Results from animal studies are controversial. We found something on CD1 mice- a stock characterized by John Ashby as not quite suitable for estrogenicity studies. As far as I know, the Vom Saal team intended using just these mice for BPA studies. And other groups used CD1 mice as well. Their heterozygosity enables an explanation why we found sterile males in two consequent generations but not in the third one. The cause could be linked with water absorption dependent on Ers in epididymis (Hess et al, Nature '97). We found (Peknicova et al 2002) decreased acrosomal reaction of epididymal sperm. Why lower dose had more pronounced effect compared with the higher dose I really do not understand."

Where it is Being Found

According to a 1999 report about 65% of the bisphenol A produced is used to make polycarbonate, and approximately 25% is used in epoxy resin production. The remaining 10% is used in other products such as specialty resins and in the manufacture of flame-retardants, such as tetrabromobisphenol A.6 Ultimately about 2 million metric tons of BPA make its way into circulation every year, and only a small fraction of that is recycled. What happens to the rest?

Researchers are finding BPA and its derivatives in a variety of sources including foods, food containers, and dental sealants. In theory, most of the BPA remains bound up in plastics or epoxies, where it remains unavailable to people and animals. However, recent research by Koehler et al. 2003 has suggested that harsh detergents can leach BPA from polycarbonate lab cages.7

Experiments have suggested that once BPA is consumed it is readily broken down into a non-estrogenic form by the liver.8

On the other hand, researchers are finding BPA and its derivatives in human blood, embryos, amniotic fluid, and the environment. Industry questions whether the levels found pose a substantial risk, while environmental advocates maintain that the levels being found in humans and environment are significant and pose a human and environmental hazard.

Oral Intake

The most likely human exposure route is through foods and beverages. This could be a result of some step in the production process, leaching from the polycarbonate bottles, epoxy can liners, or recycled paper products.

The Association of Plastics Manufacturers in Europe (APME) has calculated that under worst-case conditions, the maximum intake of BPA from food cans is approximately .8 ppb/ bodyweight/ day.9

Research by Takao et al found a maximum BPA level of 127 ppb in coffee, while the next highest concentration was only 10 ppb for tomato juice. The coffee was subsequently reformulated to contain lower levels.10

Concerns about food containers

BPA is being found in food and food containers at varying levels. Some of various food container sources include polycarbonate water containers, baby bottles, food and beverage cans, and disposable paper food containers.

Polycarbonate containers and baby bottles

Baby bottles pose one of the biggest potential concerns for BPA researchers since the target population (infants) is likely to be at highest human risk from estrogen mimicking chemicals.

These concerns first arose after a 1993 study by Krishnan and colleagues at Stanford Medical School reported how polycarbonate laboratory flasks heated to 121 degrees C for 25 minutes released 2-5 ppb of bisphenol A into water-filled flasks.11

Research by Arizono and associates found that

up to 6.5 ppb leached from old polycarbonate baby bottles heated up to 95 C for 30 minutes, but new bottles only leached up to 3.5 ppb. Bottles from outside of Japan had even higher leach rates. Arizono found that scratched bottles from the Philippines leached approximately 30 ppb and those from Korea leached over 15 ppb, more than 5 times the amount leached by new bottles.12

A World Wild Life summary paper13 published by Gwynne Lyons mentions an unpublished study commissioned by the UK Government Department of Trade and Industry which found BPA leaching out at 10-20 ppb of BPA in used baby bottles subject to brushing and/or dishwashing and sterilization, and one value even recorded a level of 50 ppb. It was not found in the liquid from new baby bottles.14

A study of the water stored in 5-gallon polycarbonate for 39 weeks found BPA levels ranging from zero to 5 ppb.15 A range of studies by industry and governments were unable to detect significant levels of BPA from polycarbonate including one by the Society of Plastics Industry (detection limit 5 ppb), and by the UK Ministry of Agriculture (detection limit 30 ppb).16

Cans

Brotons et al. (1995)17 reported BPA was found in the liquor in some cans of tinned vegetables. The highest levels were found in some cans of peas with an average of 23 ug per can. It was also found in cans of artichokes, beans, mixed vegetables, corn and mushrooms. There was no detectable bisphenol A in cans of palm hearts, asparagus, peppers and tomatoes. These liquors were found to be estrogenic, while the cans without BPA were not.

A study by Biles et al (1997)18 found that autoclaving appeared to leach BPA from milk, pork & bean, and concentrated infant milk formula cans into water. All canned foods are normally autoclaved after canning. Some questions remain about the implications that BPA leaches into all food cans, considering it was not detected in some of the Brotons samples. The levels of BPA found varied between .1 and 13.2 ppb, which is one to six times lower than in the Brotons study.

A 2001 UK Food Standards Agency study on the leaching of bisphenol a from food cans found BPA levels at up to 70 ppb in 37 samples, and at 350-420 ppb in one sample.19 The independent Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) concluded that the levels of BPA identified in canned foods analyzed in this survey are unlikely to be of concern to health, and that there is no reason for consumers to change their source of foodstuffs as a result of these findings.

In a Japanese NIH study of BPA in various drinks Kawamura et al (1999)20 reported BPA concentration and detection frequency in coffee, black tea and other tea drinks at 3.3 to 8.5 ppb.

A UK Food Standards Agency study21 found BPA in a variety of sources. A value of 420 ppb was found in one sample of meat. No BPA was detected in beverages or infant formulas. Based on the mean contaminant level the intake of BPA for the 97.5th percentile consumer of canned foods (eating 1.05 kg/day for adults and 0.38 kg/day for infants) was estimated to be 0.37 ppb bodyweight/day for adults and 0.85 ppb bodyweight/day for infants.

A 2001 EU Risk Assessment Report on BPA estimated the average daily intake of BPA at up to .37 ppb/day for adults and .85 for infants.22 It noted that taking a more realistic worst-case migration level of 10 ppb and the highest ratio of food intake to body weight for infants (a 4.5 kg infant consuming 0.7 liter of formula each day), an intake of 1.6 ppb of bodyweight per day can be estimated.

Other food containers

The Asahi Shimbum newspaper reported on a recent study by Ozaki et al that detected BPA in 13 out of 16 items made from virgin pulp at levels ranging from 34-360 ppb.23 The Osaka City Institute of Public Health and Environmental Sciences conducted the study. BPA levels between 190 and 26,000 ppb were found in eight of the twelve food containers from recycled paper. Microscopic traces of ink and copy paper were found in items made from the recycled pulp. BPA has been used as an ingredient in thermal fax paper, which might explain the relatively high levels found in some

recycled containers.

Ozaki was quoted as saying, "The levels (detected in the tests) aren't so high they could be a direct threat to human health, but there should be some sort of guideline for paper products."

Dental Sealants

Samples of the saliva from 11 patients taken 1 hour after dental treatment contained bisphenol A and bis-GMA (bisphenol A diglycidyl ether methacrylate (bis-GMA).24

There is some dispute about the details of this research.25, 26, 27

In a later study from Olea's laboratory, samples of composites and sealants polymerized in glass dishes were extracted with water of varying pH for twenty-four hours. Low levels of BPA (< 1 µg BPA/mg sealant) were reported for these materials.28

Atkinson et al (2002) 29 have suggested that the analytical method used by Olea may not have been capable of distinguishing between BPA and TEGDMA, which is known to be a predominant component released from dental sealants but not reported at all by Olea. They maintain the maximum amount of BPA that could reasonably be released from the dental sealant has been estimated to be less than the lowest level reported by Olea. Consequently, TEGDMA may have been misidentified as BPA in the Olea study.

Imai wrote in an EHP letter26 on the Olea report24 "Olea et al. reported that 90-931 µg of BPA was identified in saliva collected from 18 subjects treated with 50 mg of a bisphenol A diglycidyl methacrylate (bis-GMA)-based sealant on molars during a 1-hr period after treatment. However, based on data presented in their Table 1, this is unlikely. According to Table 1, 50 mg of the sealant should contain 3.7 µg of BPA or 78.7 µg of a mixture of bis- GMA, bisphenol A diglycidyl ether (BADGE), bisphenol A dimethyacrylate (BPDMA), and BPA. This mixture (78.7 µg) is equivalent to 48.2 µg of BPA. Assuming that all the components of the mixture leached into the saliva uncured and were degraded completely to BPA within 1 hr, the amount of BPA collected from the saliva should be 48.2 µg. However, their Table 2 showed 1.9-19.3-fold higher values than expected (89.9-931.0 µg)."

Imai concluded, "BPA and BADGE are not components, but only a trace amount of these materials is contained as impurities in bis-GMA and/or BPDMA monomers. Therefore, the amount of leached BPA is extremely small or not detectable, as reported recently by other investigators. On the other hand, BPDMA is a component of the sealant used in their study. Therefore, leaching of a small amount of unpolymerized BPDMA is likely. Furthermore, the detection of BPA derived from BPDMA in saliva is likely because BPDMA is easily hydrolyzed to BPA in saliva. Thus, it is reasonable to assume that most of the BPA identified in saliva by Olea et al. can be attributed to leached BPDMA. Therefore, the use of BPDMA should be extensively examined.

"Data relevant to HPLC analysis presented by Olea et al. (1996)24 are not reliable. These data should be corrected or withdrawn, and further reference to this data should be avoided."

Other researchers found different results than Olea. For example, Fung et al (2000)30 reported BPA was detected more than 250 times lower than the maximum amount reported by Olea. They note that BPA released orally from a dental sealant may either not be absorbed or is not detectable at or above 5ppb when measured in systemic circulation.

The American Dental Association claims all 12 ADA approved sealants tested negative for BPA leaching, after 1 version's manufacture was changed. The test sensitivity was 5 ppb.31

Another ADA survey of 40 dentists found no BPA in any blood samples.32

Nathanson et al report in a survey of seven sealants, that none of the seven sealants showed detectable amounts of BPA after extracting with ethanol with a detection limit of 0.0001 µg BPA/mg sealant.33

Olea responded27 to the concerns, "Imai seems to be upset about our recommendation to curtail the use of bisphenol A-based sealants and claims to have found unscientific data among our HPLC results. His opinions appear to be based on a poor reading of our study and what he refers to as "relevant papers." Unfortunately, Imai reveals no

data of his own and reports no experiments or time spent actually trying to solve this problem.

"The work by Nathanson et al. (1997) was largely devoted to refuting our paper on the estrogenicity of pit and fissure sealants. Their claim that their findings on leachability contradict our results implies that the two studies are comparable, while in fact their study differs substantially from ours. First, Nathanson's paper does not test the estrogenicity of sealant eluates, although the demonstration of estrogenic compounds leaching from a sealant was the most important finding of our study, a point that was also ignored by Imai. The nature of these compounds could be diverse because several components of commercial sealants have been identified as estrogenic xenobiotics. We postulated that bisphenol A and the dimethyacrylate of bisphenol A (bisDMA) are candidates for this hormonal effect, but the presence of other chemicals contributing to this effect cannot be ruled out."

Olea concluded, "We think that more data should be gathered before adopting the complacent position proposed by Nathanson et al. (1997) and Imai (1999). Recent observations have raised concerns about the estrogenicity of bisphenols: a) a more potent in vivo effect of BPA than that assessed by previous in vitro assays has been demonstrated; b) BPA seems to act on target organs other than breast and uterus; c) genetic differences in susceptibility to the estrogenic effect of BPA have increased concerns about human subpopulations with a higher sensitivity to this estrogen; and d) bisphenol A is just one of many compounds used by the plastics industry with demonstrated in vitro estrogenicity. By taking these studies into account, the American Dental Association may consider adopting a more realistic position regarding the use of bis-GMA-based composites and sealants, as recently suggested by Soderholm and Mariotti.

"In short, the flaws in the paper by Nathanson et al. (1997) undermine its conclusions on the safety of sealants; we regret the JADA Editor-in- Chief's decision not to publish our rebuttal to the allegations of Nathanson's group. Imai should not have used these flawed data to support his attack on our work. Dental health professionals ought to be aware of the potential risks of hormonally active compounds present in these formulations. Suppliers and other stakeholders should also be encouraged to address this public health issue. Until data are provided that challenge our data and arguments, the hypothesis of human exposure to estrogenic compounds leaching from bis-GMA-based composites cannot be withdrawn."

Olea also noted that the Journal of the American Dental Association refused to publish a letter critiquing the Nathanson study, despite ever-shorter versions. The implication being that the JADA did not want to take a balanced approach to exploring this potential health concern.

Human Exposure

The greatest area of concern around BPA involves exposure by fetuses and infants, a population, which is likely to be the most sensitive to hormone modulators. Takahashi et al. (2000)34 reported that absorption and distribution of BPA in maternal organs and fetuses are extremely rapid and that the placenta does not act as a barrier to BPA. Fetal levels peaked 20 minutes after the parent was orally exposed to BPA. However, clearance was equally rapid. The levels of BPA in maternal blood were only 2-5% of the maximum after 6 hours.

Dr. Pete Meyers wrote35 about this study, "The rapidity and efficiency of transfer has significant implications for epidemiological studies of bisphenol A's impact in humans. It implies that single exposures, for example via high rates of leaching from a food container marketed as usable in microwave ovens, could quickly but ephemerally pass into the mother, into the fetus, and after lingering for several hours disappear. If the passage took place during a critical, vulnerable window of development and provoked an effect, the impact would be virtually impossible to link statistically to the exposure via current epidemiological practices. This ephemerality of the contaminant coupled with its high potency during passing windows of development are important reasons why human epidemiology is strongly biased toward false negatives (concluding a product is safe when in fact it causes harm) in the study of endocrine disruption."

Miyakoda et al. (1999)36 reported the concentration of bisphenol A in both maternal blood

plasma and fetuses peaked within 1 h after oral administration. At 3 h, the concentration of bisphenol A in maternal blood plasma had decreased to approximately 10% of the peak value. The 3-h decrease in fetuses was only about 40% of the peak, and by 24 h, the fetal concentration had increased again to the nearly 70% of the peak value. The results suggest that bisphenol A might easily pass through the placental barrier, unlike sex hormones such as estrogen. A subsequent report by Miyakoda et al (2000)37, suggests that the main BPA metabolite, BPA glucuronide does not easily pass through the placental barrier. However, BPA and BPA glucuronide does appear to migrate into the testis.

In a study involving the s.c. injection of BPA into pregnant monkeys, Uchida et al, (2001)38 found that BPA was found in maternal and fetal sera, liver, brain, placenta, and fetal uteri and testes as early as 30 min after injection. They also found BPA in human umbilical cord (0.85-3.11 ng/g wet tissue; Takada et al., 1998). BPA was found in all organs investigated including fetal liver, kidney, brain and umbilical cord. These results indicate that the maternal placental barrier cannot protect the fetus from the consequences of direct BPA exposure.

In a study of BPA contamination in humans Ikezuki et al. (2002)39 reported BPA was present in serum and follicular fluid at approximately 1-2 ppb, as well as in fetal serum and full-term amniotic fluid, confirming passage through the placenta. Surprisingly, an approximately 5-fold higher concentration, 8.3 +/- 8.7 ppb, was revealed in amniotic fluid at 15-18 weeks gestation, compared with other fluids. They concluded, "These results suggest accumulation of BPA in early fetuses and significant exposure during the prenatal period, which must be considered in evaluating the potential for human exposure to endocrine-disrupting chemicals."

A study conducted in Japan by O. Takahashi et al. (2000)40 on rats showing that the placenta does not serve as a barrier to transmission of BPA, suggests that parent BPA can be transferred from the human mother to the fetus via the placenta. Although parent BPA is rapidly eliminated by first-pass metabolism following oral administration, parent BPA can be found in human placental tissue following normal food consumption.

This is particularly interesting because the parent BPA compound is thought to rapidly breakdown into BPA monoglucuronide, which is biologically inactive. In 14 of 37 parental-fetal subjects the fetal levels of BPA were higher. It was suggested this might be due to the lack of enzymes capable of breaking down BPA. The authors noted, "Further studies on human exposure to BPA are needed to address the question whether maternal exposure to BPA can lead to adverse health effects in offspring."

Schönfelder et al. (2001)41, (2002)42 reported concentrations of BPA ranged from 0.3 to 18.9 ppb (median = 3.1 ppb) in maternal plasma, from 0.2 to 9.2 ppb (median = 2.3 ppb) in fetal plasma, and from 1.0 to 104.9 ppb (median = 12.7 ppb) in placental tissue. BPA blood concentrations were higher in male than in female fetuses.

In a 10-year study of 200 pregnant Japanese women, Yamada et al (2002)43 reported that median BPA concentration in maternal serum over a ten- year period was 2.24 ppb ranging from 0.63 to 14.36 ppb. Median BPA concentration in amniotic fluid was 0.26 ppb, ranging from 0 to 5.62 ppb. Eight of 200 fetuses were surrounded in amniotic fluid containing a relatively high concentration (2.80-5.62 ppb) of BPA. BPA levels were higher in the blood of mothers in seven of these eight fetuses.

Once BPA enters the body, some studies44, 45suggest it is rapidly transformed into BPA monoglucuronide, which does not compete with estradiol and unlike BPA does not cause estrogen receptor gene expression n MCF-7 cells or HEPG2 cells.

Other metabolites and BPA related compounds, which are not estrogenic of themselves such as Bis-GMA and BADGE could be transformed into active compounds in acid or alkaline media.46

Human /Animal Comparisons

One of the main difficulties in extrapolating the effects of BPA from rats and mice to humans involves the differences in metabolic pathways between the two species. Elsby et al (2001)47 reported a 3/7 ratio of human to rat liver microsomal metabolism. It has been suggested the human fetus

may have even less ability to make this conversion because the metabolic pathways does not develop until after birth. The authors "suggest that assessment of the estrogenicity of BPA using the immature rat uterotrophic assay might well underestimate the potency of BPA in humans."

However, although each rat liver cell appears to be more efficient than those of humans, Pritchett et al. (2002) suggests humans are likely to be more efficient at breaking down BPA due to a relatively larger liver.48

Another issue is the relative liver size. Witorsch (2002)49 noted, "An issue that is potentially relevant to the health implications of low-dose xenoestrogen exposure in utero, and not previously addressed, is the comparative physiology of gestation in the mouse and human. These two species differ with regard to the extent of involvement and hormonal control of the corpus luteum, organs involved in progestin and estrogen secretion, the specific estrogens produced, and estrogen blood levels attained in the mother and embryo. On the basis of these species differences (particularly, the markedly higher estrogen levels attained in human pregnancy compared to the mouse), it would appear unlikely that low doses of BPA or other xenoestrogens produce adverse endocrine disruptive effects during human pregnancy."

Major differences on the effects of BPA and other estrogenic compounds have been observed within genetic variants of rats. For example, Long et al (2000)50 report BPA stimulated the secretion of the brain hormone that initiates lactation (prolactin) in Fischer 344 rats but not in Sprague-Dawley rats.

Spearow et al. (1999)51 report CD-1 mice appear to be 16 times less responsive to estradiol than some other strains. It may be possible that subtle genetic difference may explain how researchers like Vom Saal are finding low-dose effects while others are not.

BPA In the Environment

Another set of concerns around BPA involves its migration into the environment, particularly water streams. Kolpin et al (2002)52 report that approximately 60% of the streams contained no detectable level of BPA (detection limit 0.09 ppb), the median detected concentration was 0.14 ppb, and only 2 streams were reported to contain BPA at levels above 1 ppb.

A Japanese Environment Agency Report53 on environmental estrogens found detectable BPA in 67 of 124 water samples. The median concentration of BPA, where detected, was 0.01 ppb and 95% of the samples contained less than 0.24 ppb of BPA.

Staples et al. (2000)54 report that an initial BPA survey did not find BPA in the waters was upstream or downstream of five BPA factories and two processing sites tested with a detection limit of 1 ppb. A follow up study found BPA at levels ranging from 2-8 ppb upstream and 7-8 ppb downstream of one BPA factory. Some BPA was apparently present in the discharged effluent and likely flowed back into the area where the upstream samples were collected.

Rippen et al (1999)55 report finding BPA in rivers at levels ranging from under .005 to 1.9 ppb in the water and at levels ranging from 5-100 ppb in the stream sediment.

Klecka et al, (2001)56 have suggested that biodegradation plays a major role in the removal of BPA from the environment. They report BPA degrades rapidly in surface waters and sediments taken from a wide variety of geographies (including those with no known exposure history), suggesting that microorganisms with the capability to degrade BPA are ubiquitous in the environment. It was noted that studies using real world surface water samples demonstrated a half-life of 1-4 days of BPA.

Studies57 using real world surface water samples taken from various geographies demonstrate rapid degradation with a half-life in the range of 1 to 4 days (i.e., time for 50% degradation). A Cousins et al, (2002)58 survey reported the median reported water concentrations from 21 European and 13 United States studies are 0.016 and 0.5 ppb respectively. In cases where individual concentration data are reported, many samples have no detectable level of BPA.

BPA Effects

A wide variety of effects have been reported

from relatively low-dose exposures to BPA, at levels comparable to what is being found in human and fetal blood in both in-vivo and in-vitro studies. The in-vitro reports are interesting from a mechanistic point of view. But in-vivo reports, which suggest the living organisms in their entirety are affected, are more toxicologically relevant for health assessment. Despite the growing body of in-vivo BPA low-dose findings none have been successfully replicated across multiple labs, and only one, Sakaue et al, 2001, has been repeated within the same lab. This may explain the reluctance for policy regulators to take any action on BPA at this time.

This survey highlights some of the more interesting low dose effects reported in the literature.

BPA In Vivo Effects On Rats

20 ppb: +epididymis & seminal vesicle weight (Fritz, 1999)59

This study, reported in abstract only reported on a study in which male Sprague-Dawley CD rats were exposed to 20 and 200 ppb bisphenol A per gram diet from conception until day 70 postpartum. Adult animals were found to consume 2.2 and 23.1 ppb bodyweight, respectively. Both groups had significantly reduced bodyweight compared to controls. The seminal vesicles and epididymis were increased in weight in a dose-dependent manner, and kidney and spleen weights were decreased for both concentrations. The SCF BPA report noted that "other the other effects reported in the same study, of reduced body weight, kidney and spleen weights at these low doses, are not congruent with other studies on BPA."

20 ppb: Sperm and testes effects (Sakaue 2001)60

In this study male rats were administrated a daily oral dose of BPA, ranging from 2 to 200,000 ppb. A dose as low as 20 ppb tended to decrease testicular weight and significantly reduced both daily sperm production and the efficiency of spermatogenesis. These effects were found in rats administered BPA after puberty, which is interesting because adults are thought to be less sensitive to xenoestrogens than infants.

The SCF BPA report noted the size of the effect (25-30% reduction in daily sperm production and efficiency of spermatogenesis) and the flat nature of the dose-response are compatible with the lack of any functional consequence for fertility in that species, recorded in other studies. Other labs have been unable to replicate these findings. Consequently the SCF has concluded, on the present weight of evidence, that the reported findings on juvenile/adult sperm parameters from one laboratory should not be considered a pivotal effect in risk assessment of BPA.

But John Ashby said his team failed to confirm this effect in 5 repeats.

20 ppb premature vaginal opening (Freien Universitat)61, 62, 63,64,65,66,67

Chahoud et al reported in a series of articles that exposure of pregnant Sprague-Dawley rats delayed vaginal opening at 20 or 100 ppb bodyweight/day (but premature vaginal opening at 50,000 ppb bodyweight/day) and an increase in prolongation of estrus by more than 1 day at 100 ppb/day. They suggest that the loss of ER alpha expression in the vagina during the estrus stage leads to these morphological findings.

None of the results could be replicated in either Sprague-Dawley rats or in another rat strain by another lab (Tinwell et al, 2002).68 The Chahoud study has been criticized by the National Toxicology Program's statistic subpanel for the lack of concurrent controls since treatment effects may be confounded by time-related changes (the controls were studied sequentially). The NTP noted, "The lack of concurrent controls in this study was a serious design deficiency. This confounding of possible treatment effects with time-related changes precludes any reliable assessment."69

25 ppb: Differentiation pattern of periductal stromal cells of the ventral prostate affected (Ramos et al, 2001)70

Ramos et al exposed fetal mice to BPA using an osmotic pump implanted in pregnant rats to doses of 25 or 250 ppb. They found significant alterations in the development of the rat prostate. Some of

these changes resemble human prostate conditions that enhance the potential of a nascent prostate cancer tumor. The exposure increased the expression of the protein vimentin at all doses. The percentage of prostate stromal cells with androgen receptors also appeared to decrease with BPA exposure. Grossfield et al, 1998, report alterations in the stroma appear to enhance the invasive or malignant potential of a new tumor. No epidemiological studies have been conducted exploring the human link between BPA and prostate cancer.

40 ppb: Behavioral Effects71

Researchers gave female Sprague-Dawley rats 40 ppb BPA from conception to weaning postnatal day 21 and 400 ppb/day BPA from gestation day 14 to postnatal day 6. After exposure, they studied social behavior in a play situation in juvenile male and female offspring. They observed an early action of BPA on several behavioral categories in both males and females. In particular they observed a masculinization of female behavior in two behavioral categories (play with females and sociosexual exploration), an effect probably mediated by the estrogenic activity of BPA in the CNS. The authors suggest these long-lasting effects of BPA could have important consequences at individual and population levels.

In a separate report, Farabollini et al, (2002)72 report that reported an increase in defensive behavior in males, but not females. Sexual performance impairment was noted in terms of latency and frequency of intromissions. BPA was correlated with increased sexual motivation and receptive behavior in females.

In a personal communication, Dessì-Fulgheri noted submitting a study with F Farabollini on the effects of BPA on maternal behavior in rats, and with M Canonaco on the effects of BPA perinatal administration on somatostatin receptors sst3. While these results are fascinating, and raise some profound implications regarding BPA effects on human behavior, this line of research does not seem to be considered by mainstream toxicologists in setting exposure limits.

Adriani et al (2002)73 report that female rats orally administered BPA at 40 ppb spent significantly less time than did controls in novelty exploration, whereas no effect was found in the male group. In addition, a reduced level of impulsive behavior was evidenced in BPA-treated rats. The authors suggest "these findings provide clear indirect evidence of long-term alterations in brain monoaminergic function after perinatal BPA exposure. This may be a cause for concern for public health, confirming that exposure to a weak environmental estrogen in the period of sexual differentiation of the brain can influence adult behavior."

100 ppb: Increase in body weight (Rubin, 2001)74

Reported effects of orally delivered BPA at levels of approximately 100 ppb bodyweight/day to pregnant rats. They found that BPA exposed rats were heavier than control animals at birth. They also found that 1200 ppb bodyweight/day exposed animals appeared to have a defect in the pattern of estrus activity and lower plasma LH levels.

100 ppb: Breast Effects (Colerangle, 1997)75

They found that the conversion of immature structures to mature structures was significantly increased in response to exposure to both low (100 ppb/day) and high (54,000 ppb/day) doses of BPA compared to controls. The proliferative activity of epithelial cells was increased by 143% over controls by the exposure of animals to the low dose of bisphenol A, whereas a 220% increase over controls was observed for the high dose of BPA. The authors note that the weak estrogenic activity of BPA does not explain its profound effect on cell proliferation observed in this study. Perturbation of the cell cycle is considered a risk factor for the development of cancer.

It should be noted however that Ashby et al have raised serious concerns about the methodology employed in these studies based on their own attempts to confirm effects reported by Colerangle and Roy.

100 ppb Reduction in testis weight and sperm production in adulthood following single

maternal dose (Sharpe, 1996)76

One study reported a reduction in testis weight and sperm production after a single 100-400 ppb maternal exposure. Subsequent studies by the same authors reported shifts in the same testicular parameters in controls.77 These findings were not replicated by a variety of industry-funded studies.

This study could not be replicated by Cagen et al (1999)78, or Ashby et al (1997)79

100 ppb reduced sperm content (Bowers, 2001)80

Found that male rats given 100 or 250 ppb BPA orally on days 1-14 postnatally had lower sperm content in the testis and epididymis at 44 days. It was only published in abstract form.

100 ppb: affects expression of Estrogen Receptor alpha. (Schönfelder 2002)81

Found that the full-length ER alpha is not expressed during estrus in the vagina of female offspring exposed to either dose of BPA when compared to the control group, whereas ER alpha expression does not differ from the control group during the diestrus stage. ER alpha downregulation seems to be responsible for the observed altered vaginal morphology.

BPA Effects on Mice

2 ppb: Increased Aggression; Lower Testis Weight (Kawai 2003)82

Evaluated effects of BPA on aggressive behavior and hormonal change in male mice fed 2 and 20 ppb. Aggression scores increased significantly ($p < 0.01$) at 8 weeks of age in male mice exposed to bisphenol A at both the 2 ng/g and 20 ng/g concentrations compared with a control group, but no difference was found after 12 weeks. Relative testis weight (per gram of body weight) was significantly lower at 8 and 12 weeks in mice treated with 2 ng/g than in controls ($p < 0.05$) and was significantly lower at 12 weeks in mice treated with 20 ng/g than in controls ($p < 0.01$). Suggests that bisphenol A temporarily activated aggressive behavior in mice at 8 weeks of age and that low doses of bisphenol A interfered with the normal development of reproductive organs.

2 ppb: Increase in prostate & preputial gland weight. Decreased daily sperm production & epididymal weight (Nagel 1997)83, (vom Saal 1998)84

This is the study that galvanized the debate over the possible low-dose effects of BPA. The study has been criticized for its small size and the lack of repeatability.

This same team has also reported in abstract only increased prostate size in another strain of mouse after prenatal exposure to 10 ppb bodyweight/day orally. (Vom Saal 2000.)85

In a subsequent paper (Howdeshell 2000)86, these researchers report that intrauterine position (IUP) can affect susceptibility to the effects of BPA. They noted that fetal exposure via the mother to bisphenol A increased the rate of postnatal growth in males and females and also advanced the timing of puberty in females. However, the greatest response to bisphenol A occurred in males and females with the highest background levels of endogenous estradiol during fetal life due to their intrauterine position, while fetuses with the lowest endogenous levels of estradiol showed no response to maternal bisphenol A treatment.

Other scientists have tried unsuccessfully to repeat these experiments. 87, 88

2.4 ppb: Increase in body weight; advance in age of 1st estrus (Howdeshell 1999)89

The same vom Saal team reported that female mice exposed to BPA in the womb had a reduced delay between vaginal opening and first estrus.

Honma et al. (2001)90 attempted to repeat this study. They found that in all DES- and BPA-treated groups, body weight on day 22 was significantly lighter than controls. The age at vaginal opening was significantly earlier in all treated groups except for 2 ppb BPA-treated group compared to controls. Body weight at vaginal opening was lighter in all treated groups than in controls. The first vaginal estrus was also earlier in all treated groups than in

controls. Total estrous days in all DES- and BPA-treated groups were longer than in controls. Their results were different from those of Howdeshell et al, (1999); body weight at weaning was lighter (different), age at vaginal opening was earlier (different) and age at the first estrous was earlier (same). These results indicate that prenatal exposure to low doses of DES and BPA accelerate vaginal opening, however, weight gain could not be confirmed.

Ashby et al. (1999)88 in a similar experiment using 2 or 20 ppb BPA confirmed the absence of any effect on timing of vaginal opening but did not examine time of first estrus and did not find any increase in female weaning weight.

4 ppb: luminal epithelial cells of the endometrium taller and microvilli longer; vaginal epithelium had increased; enlargement of the nuclei and widening of the intercellular space among the stratified cells (Fukumori, 2001)91

Mice were treated with .8, 4, and 20 ppb / day BPA by s.c injection. At 4 ppb they reported the luminal epithelial cells of the endometrium showed taller cell height than those of the control. Microvilli of these cells also tended to be longer than those of the control. They also showed increased cell layers (5-7 versus only 3-4 in the control).

10 ppb: Fertility effects (Buckiova, 2001)92

These researchers observed damage of in vivo fertility, reproductive organs and spermatogenesis after BPA treatment. They also noted that effects on the reproductive system in offspring, predominantly in 2nd generation. Beside decreased litter size in BPA treated groups they observed sterile pairs of mice in every generation. No size changes were noted. They noted that the data from this study indicates that effects of BPA do not follow a classical dose - response curve since the effect of lower dose was more pronounced.

Buckiova wrote, "Enclosed please find more information about our BPA research on CD 1 mice. The first attached document93 is referring to using a test of the acrosome reaction for detecting sperm damage after BPA treatment. Test of the acrosome reaction means that a capacitated sperm (epididymal) is able to undergo the acrosome reaction in response to contact with the zona pellucida. Detection of the acrosome was made with the fluorescent microscopy. It is one of parameters used for the assessment of the fertility status.

"BPA effects on fertility CD 1 mice were compared with NP, GEN and DES treatment.94 Referring to BPA treatment we observed decreased testosterone levels in blood of 6 weeks old males. Beside decreased litter size we observed sterile pairs of mice in every generation. Testis histopathology correlated with fertility in vivo and observed defects in spermatogenesis, including pathological state of sperm."

10 ppb: Maternal behavior affected in mice exposed either in utero or during adulthood (Palanza 2002)95

20 ppb: Advance in age at vaginal opening and at 1st estrus (Honma, 2002)96

20 ppb: increase in chromosomal aberration (Hunt, 2003)97

They found that BPA was leaching from polycarbonate cages and drinking bottles, causing genetic defects in exposed mice. Mice were treated with 20, 40, or 100 ppb bodyweight. The rate of congression failure, a type of chromosomal aberration increased significantly at the lowest dose. They conclude, "Clearly, the possibility that BPA exposure increases the likelihood of genetically abnormal offspring is too serious to be dismissed without further study."

The American Plastics Council's bisphenola.org website wrote98 about this study, *"Reproductive and developmental effects were not examined in the new study and the experimental system used has not been validated or standardized for the evaluation of reproductive effects. Indeed, the authors of the study note that the relevance of the reported results to human health has not been established. The potential for BPA to cause reproductive or developmental effects has been comprehensively examined in two multi-generation studies. In these studies*

BPA did not cause reproductive or developmental effects at any environmentally relevant dose. The weight of scientific evidence provided by these studies, as well as others that have looked specifically at reproductive or developmental effects, clearly supports the safety of BPA and provides strong reassurance that there is no basis for human health concerns from exposure to environmentally relevant doses of BPA...

"Although the paper might be read to suggest that BPA or other environmental chemicals could have an effect on reproduction or development, no direct evidence to support this suggestion is reported in the paper since the experimental results did not examine reproduction or development. Indeed, as noted by the authors, additional research is needed to determine if their experimental system might provide a sensitive, reliable, and reproducible assay system for the evaluation of reproductive toxins. At this point, the experimental system has not been validated or standardized for use in assessing potential risk to human health. Consequently, as further noted by the authors, the relevance of the reported results to humans has not been established.

"Environmentally Relevant Doses of BPA Do Not Cause Reproductive or Developmental Effects

"Since the Hunt et al. study did not examine reproduction or development in their laboratory animals, additional research is needed to determine if the reported chromosomal abnormalities actually lead to functional effects. Although not referenced in the paper, extensive research has already been conducted to answer precisely this question.

"Most notably, this includes a three-generation reproduction and development study on BPA conducted at the Research Triangle Institute under the direction of Dr. Rochelle Tyl,99 which is one of the most comprehensive studies of its kind ever conducted. In this study, Sprague-Dawley rats were fed a diet containing BPA at levels lower than those tested by Hunt et al. and ranging to levels more than a thousand times higher. A wide range of endpoints was examined to determine if BPA had any effect on the reproductive performance of the animals or on the development of the offspring. Analysis of the data for all of the endpoints for the parental and three offspring generations revealed no evidence for reproductive or developmental effects at any environmentally relevant dose. This exceptionally powerful study, which complied with Good Laboratory Practice standards and was conducted in accordance with internationally accepted guidelines, provides a definitive conclusion that BPA does not cause reproductive or offspring effects at low doses. Additional information on this study is available at http:// www.bisphenol-a.org/development/whatsNew/ 20020702DefinitivePeer.html.

"The results of a similar two-generation study commissioned by the Japanese National Institute of Health Sciences fully support the conclusions of the three-generation study.100 "In this study, which also covered doses ranging from below to above the doses tested by Hunt et al., no effects on reproduction or development were found at any dose. In addition, this study also examined the offspring in two behavioral tests, including a learning test, and found no effects from exposure to BPA at any dose.

"In addition, the results of a continuous breeding study in mice, conducted by the U.S. National Toxicology Program,101 showed no effects on reproduction at a dose approximately 1000 times higher than the highest dose tested by Hunt et al.

"The weight of scientific evidence provided by these studies clearly supports the safety of BPA and provides strong reassurance that there is no basis for human health concerns from exposure to environmentally relevant doses of BPA. Additional information on the weight of evidence is available at http:// www.bisphenol-a.org/pdf/LowDoseUnprovenOctober2002.pdf (33kb, PDF).

"Consistent with this conclusion, the use of polycarbonate plastic and epoxy resins for food contact applications has been and continues to be recognized as safe by the U.S. Food and Drug Administration, the European Commission

Scientific Committee on Food, the United Kingdom Food Standards Agency, the Japanese Ministry for Health, Labor and Welfare, and other regulatory authorities worldwide."

25 ppb implant Increased mammary gland maturation (Markey, 2001)102

Mammary glands of BPA-exposed mice showed differences in the rate of ductal migration into the stroma at 1 mo of age and a significant increase in the percentage of ducts, terminal ducts, terminal end buds, and alveolar buds at 6 mo of age.

Female mice were exposed in utero to BPA via osmotic pumps at doses of 25 and 250 ppb bodyweight of the mother. The 25 ppb group showed a "greater ductal elongation beyond the edge of the lymph node. The higher dose group showed retarded growth. Both groups also have lower rates of DNA synthesis compared to controls.

The researchers noted, *"In the present study, two findings may suggest a predisposition of the BPA- exposed mammary gland to neoplastic change. The altered relationship in DNA synthesis between the epithelium and the stroma that was observed at all stages of development is striking, because disruption of the communication between these two tissue compartments is acknowledged as being critical to the development of neoplasia within both human breast and rodent mammary gland. In addition, the significant increase in terminal ducts and terminal end buds in the 6-mo-old mice is also remarkable, because an increase in these structures in rats and humans correlates positively with the incidence of carcinomas that arise specifically from such sites."*

"In summary, in utero exposure to low, presumably environmentally relevant doses of BPA changes the timing of DNA synthesis in the epithelium and stroma of the mammary gland, resulting in an histoarchitecture that is atypical for a virgin mouse. These changes, which are apparent long after the period of exposure is over, strengthen the hypothesis that in utero exposure to environmental estrogens may predispose the developing fetus to mammary gland carcinogenesis in adulthood."

25 ppb s.c. decrease in fetus number and male ratio lower (Hase, 2001)103

This study examined the influence of s.c injected BPA on fetal rats at doses of 25, 2500, 25,000, and 250,000 ppb bodyweight / day. At the lowest dose, they reported a delay in vaginal opening, and a decrease in the fetus number. The male ratio was also reduced from 52.5% for the control to 40.5% for the 25 ppb groups. The ratios recover by BPA dose-dependently. These results suggested that low-dose BPA exposure prenatally affected not only puberty of the female fetus but also reproductive ability.

50 ppb Increased AGD & prostate weight; decreased epididymal weight (Gupta, 2000)104

This study could not be replicated by Ashby et al. (1999)105 or Cagen et al (1999)106

139 ppb Decreased testosterone; testicular morphology affected (Takao, 1999)107

2000 ppb: Number of tyrosine hydroxylase-positive neurons reduced; neuritis reduced; glutamate decarboxylase-positive neurons increased; calbindin-positive neurons decreased) (Fushiki, 2001)108

In BPA-treated animals, the number of tyrosine hydroxylase-positive neurons was shown to decrease together with reduction of neurites. Glutamate decarboxylase-positive neurons increased, whereas calbindin-positive neurons decreased in the cerebral cortex. During the fetal period, the pattern of distribution of GAP43 was different in BPA-treated mice compared to the controls, suggesting some inhibition of the neuronal differentiation in terms of growth cone.

In Vitro Effects

1 nanomolar = (approximately 0.23 parts per billion)

100 pM significantly affects granulosa cell viability (Jiping, 2002)109

.23 ppb interferes with normal gene expression (Quesada, 2002)110

This paper identified a new pathway for BPA to disrupt gene expression at levels as low as .23 ppb. The genes affected involve long-term memory, brain development and weight regulation. Of particular note is the suggestion that BPA affects an estrogen receptor on the cells surface rather than the nuclear receptor. These effects indicated that BPA is just as powerful as estrogen in activating these cell-wall receptors. The researchers exposed human pancreas cells to various substances including BPA.

.23 ppb increases a process in prostate cancer cells that renders them less responsive to the standard hormone treatment used to force prostatic adenocarcinomas into remission (Wetherill, 2002)111

The first stage of prostate cancer is generally stimulated by androgens. The common treatment for prostate cancer is to reduce the quantity of free androgens available to stimulate the tumor. Over time, the cancer can evolve into tumors, which grow independently of androgen. Wetherill et al reported that BPA appears to initiate the proliferation of androgen-independent cancer cells that do not respond to androgen reduction therapy. They studied human LNCaP cells growing in BPA concentration of .1 to 100 nanomolars. The largest effect was seen at 1 nanomolar (.23 ppb). They noted, "these data implicate BPA exposure as a potential mechanism that could facilitate the transition of prostatic adenocarcinomas to androgen independence."

Breast Cancer Promotional Effects @ 2 ppb (Krishnan, 1993)11

Krishnan et al 1993 reported BPA to be estrogenic in the human MCF-7 human breast cancer cell culture assay concentrations as low as 2-5 ppb.

1,000 ppb Egg Yolk atrophy (Akita, 2001)112, 113

After 48 hours, rat embryos cultured in 1000 ppb and 100,000 BPA. At 1000 ppb, the heart rates were lower and a pronounced atrophy of the vessel on the yolk sac

.0069 ppb (.03 nM) BPA affects thyroid transcription (Moriyama, 2001)114

BPA significantly suppressed thyroid transcriptional activities mediated in a dose-dependent manner. This is the first report of BPA disrupting the transcriptional activities of TRs as an antagonist. BPA may have varying effects at the transcriptional level on different nuclear receptors and their cofactors.

BPA and insulin causes 1300% rise in fat levels versus 150% for insulin alone (Masuno, 2002)115

1,300% vs. 150% increase in fat for insulin alone

They found that BPA modulates the action of insulin to increase the number and size of fat cells.

BPA in the environment

Another area of toxicological potential concern around BPA involves environmental effects. A comprehensive review of the literature by Staples et al. (2002)116 suggested that adverse effects of BPA only occur at concentrations of 160 ppb and above. The study focused on biologically relevant endpoints of survival, growth, development, and reproductive success. The lowest observed effect concentration ranged from 160 to 11,000 ppb, while the No Observed Effect Concentration range from 16 to 3,640 ppb. They reported that BPA has a minimal effect on the survival of aquatic organisms.

A study of Japanese medaka exposed to 2.28 through 1,820 ppb water found no effects on hatchling success and time to hatching, mortality, and abnormal behavior.117 However, the growth of the fish was suppressed with increasing concentration resulting in significant differences in total length and bodyweight at 1,820 ppb. When observed for their external secondary sex characteristics, no males were identified in the 1,820 ppb treatment. In addition, histological examination showed that 32% of fish in the 1,820 ppb group had testis-ova composed of both testicular germ cells and oocytes.

Consequently, the lowest effective concentration for the early life stage of medaka was between 355 and 1,820 ppb

A study of snails exposed to BPA concentrations of reported that 1 ppb induced a complex syndrome of alterations in female Marisa referred to as "super females" at the lowest concentrations.118 Affected specimens were characterized by the formation of additional female organs, an enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production.

Sex Ratio of Frogs

Kloas et al (1999)119 found that frog exposure to BPA at 23 ppb was correlated with a significant increase in the number of females.

In response to this report, a new study was commissioned by the BPA Environmental Research Task Group. The repeat study (Pickford et al. Chemosphere, in press) was performed at Brixham Environmental Laboratory (AstraZeneca), and incorporated a number of changes in study design in order to provide a more robust assessment of any influence of BPA on sex differentiation in Xenopus. A range of six BPA concentrations were tested (spanning and including the two tested by Kloas et al.)119 in order to investigate any concentration-response relationship.

While the original study used a single tank containing 48 larvae to each test concentration, the repeat study employed 4 replicate tanks of 40 larvae per test concentration, in order to provide greater statistical power and to estimate the experimental variability of the sex ratio endpoint. Other differences in the conduct of the study were the diet (the original diet was not available), and the exposure system.

In the original study, a semi-static system was used where water containing BPA (or solvent control) was replaced 3 times per week. Owing to the biodegradable nature of BPA, in lab systems as well as in the environment, a flow-through exposure system was used in the repeat study. This ensured that steady concentrations of BPA were maintained in the tanks throughout the study, and this was verified by regular chemical analysis.

The repeat study found no significant effect of BPA on larval growth, development or sex ratio (as assessed by gonadal morphology, as in the original study), though there was a significant female bias in frogs exposed to the positive control substance (estradiol, at the same concentration as used in the original study).

Possible explanations for the lack of effect of BPA in the Pickford study include the use of a different diet and a different exposure system. However, the degree of feminization in the positive control was identical to that in the positive control in the original study, indicating that the differences in test design did not have a significant effect on the sensitivity of the larvae to exogenous estrogens. Other explanations for the discrepancy may relate to the degree of experimental replication, and the type of statistical analysis performed. Kloas used a non-parametric test, while the Pickford study used a Chi-squared type test, which is the standard method for analyzing sex ratios.

The results of the second, more comprehensive study indicate that BPA has no effect on sex differentiation in Xenopus larvae (as assessed by gross gonadal morphology) in the concentration range 0.83 - 497 mg/L.

Questions about Effects

Why are some researchers and some labs able to find low-dose effects while others are not? Are these inherent differences between results caused by some systematic bias by scientists on either side of this debate? In the World Wildlife Fund BPA survey, Lyons cites a rather interesting study of tobacco research, finding that review articles written by authors with affiliations to the tobacco industry have been found to be 88 times more likely to conclude that passive smoking is not harmful than if the article is written by authors with no connection to the tobacco industry Wise (1998)120.

Lyons commented on this, "The experience gained with BPA, and similarly with tobacco, underlines the need to ensure that research scientists

with no industry connections are properly resourced."13

Part of the difficulty in tracking down low-dose effects is that they may be due to mechanisms not previously considered. For example, some effects may be caused by surface receptors on the cell wall, rather than the nuclear receptors. Nagel et al (2001)121 identified considerable difference in the efficacy and potency of ER ligands in the uterus when ER transcriptional activity was assayed vs. uterine weight gain. Specifically, they observed that the environmental estrogen bisphenol A was a potent agonist in stimulating ER transcriptional activity, whereas it exhibited little uterotrophic activity.

Another confounding factor may involve the cages or watering containers used to house and feed the animals. Howdeshell et al. (2003)122 report observing BPA leaching into the water contacting polycarbonate animal cages. The implications of this study throw uncertainty on hormone modulation experiments using polycarbonate cages. It may also explain the inability for some labs to find results that others are seeing.

They found that old polycarbonate cages leached the most BPA, while newer polycarbonate and polysulfone cages leached about a tenth these levels. No BPA was detected in polypropylene cages. The water from old cages exhibited estrogenic effects in the MCF-7 assay, while newer cages did not. Mice grown in the older polycarbonate cages had a prepubertal uterine wet weight 16% heavier than mice reared in polypropylene, although the results were not statistically significant.

The authors note, "Our findings here suggest that aquatic laboratory animals may be exposed to sufficient BPA due to leaching from worn polycarbonate caging to result in a significant impact on reproductive parameters. This prediction is based on a number of recent reports of significant effects at very low concentrations of BPA in frogs, fish and mollusks."

A number of possible BPA sources were cited besides polycarbonate cages. For example, BPA migration into humans has been reported with polycarbonate and polysulfone hemodialysis equipment. BPA is also added as a stabilizer in the production of PVC products.

As noted by Meyers123 an important question not addressed by the authors is how many of the BPA toxicology studies conducted thus far have used polycarbonate cages?

It is important to note that despite the plethora of research indicating low-dose effects for BPA, a number of comprehensive studies on BPA have been unable to repeat these findings. Some of these studies involved multiple generations and the evaluation of thousands of animals. Are these differences caused by some inherent bias by researchers on either side of this debate? Is this simply another manifestation of that 88-fold difference found in tobacco?

Cagen et al (1999)124 evaluated BPA effects on male sexual development in CF-1 mice at doses ranging from .2 to 200 ppb/day. There were no treatment-related effects of BPA or DES on testes histopathology, daily sperm production, or sperm count, or on prostate, preputial gland, seminal vesicle, or epididymis weights at doses previously reported to affect these organs or at doses an order of magnitude higher or lower.

Waechter et al. (1999)125 evaluated reproductive effects in male CE-1 mice exposed to BPA at doses of .2 to 200 ppb. No treatment-related effects were observed in adult females or on growth or survival of offspring from dams treated with any dose of BPA. There were no treatment-related effects of BPA on prostate weights, sperm count or daily sperm production, testis histopathology, or preputial gland, seminal vesicle, or epididymis weights in offspring at any dose of BPA. There were no effects on any parameter measured in dams or on the growth or sexual development of male offspring of dams exposed to DES during gestation. The authors concluded "those findings previously reported by Nagel et al. (1997) and vom Saal et al. (1998) were not confirmed at doses equivalent to theirs and at an order of magnitude higher and lower."

Tyl et al. (2002)126 evaluated the effects of BPA on CD Sprague Dawley rats at doses ranging from 1 to 500 ppb. They found:

Normal monotonic dose-response relationships for the few treatment-related effects observed at the

two highest doses;

No effects on reproduction or development at low doses;

No significant changes in prostate or other reproductive organ weights in any of the three generations of laboratory rats at any dose;

No effects on daily sperm production or efficiency of sperm production in any generation at any dose; and

No significant changes in markers of sexual maturity below the highest dose tested.

Dimond et al, (1999)127 Evaluated potential effects of BPA on sexual development as reported by Sharpe et al 1996. Han Wistar Albino rats were orally dosed with BPA at doses ranging from 10 to 10,000 ppb of BPA. No treatment-related effects on growth or reproductive endpoints were observed in adult females exposed to any concentration of BPA. Similarly, no treatment-related effects on the growth, survival, or reproductive parameters (including testes, prostate and preputial gland weight, sperm count, daily sperm production, or testes histopathology) of offspring from dams exposed to BPA during gestation and lactation were observed.

Why Aren't In-vivo Low-Dose Effects Repeatable

The vom Saal lab experiments have sparked the most controversy. A variety of reasons have been noted for the inability of other labs to repeat them including:

1) Technical competence;

Rebuttal: The technicians were trained by the vom Saal team.

2) Diet rich in phytoestrogens;

Rebuttal: There are higher phytoestrogen levels in the Purina diet used by vom Saal team.

3) Need to play recorded music

4) Animal pen size

5) Study too large, making it difficult for a single technician to conduct the evaluations.

6) Animals too heavy, and that as a result their reproductive tissues and functions were no longer susceptible to BPA-mediated perturbation

Rebuttal: Nonneman (1992)128 et al reported higher control prostate weights than Ashby.

Janszen et al. (2000)129 has suggested that divergence of results with regard to prostate weights is due to the natural variability of the pups within a litter

But even if these low-dose prostate effects can be repeated across labs, mainstream toxicologists may question the toxicological relevance to humans. Milman et al (2002)130 reported, "Studies conducted in our laboratories and by others found no consistent correlation between prostate size, prostate pathology, or the development of prostate cancer under a variety of experimental conditions. Furthermore, an evaluation of eight published studies that were conducted in mice and rats following in utero exposure by oral treatment of dams with low levels of bisphenol A (BPA) and that focused on the prostate identified several discrepancies that affect their adequacy for use in human risk assessment. For example, there was inadequate reporting of the purity of BPA and the animal supplier used, and housing of offspring was not the same among the studies. In addition, there were differences between studies with mice and rats in exposure regimen, route of exposure, and numbers of dams or pups used per BPA dose group. Poor inter- and intraspecies correlation (i.e., mouse to rat or between mouse or rat strains) further complicates the ability to use results from these studies to predict potential prostate effects in humans. Thus, we conclude that a finding of increased prostate weight in rodent studies with perinatal exposure in the absence of associated pathologic and/or functional changes is meaningless and not indicative of a potential adverse effect in humans."

Government Actions and

Regulation

Government Pronouncements on BPA

At the moment at least, no government has reported on cause for concern around the use of BPA in any products. There appears to be a reluctance to take any action regarding these low dose studies, owing to a perceived lack of weight of evidence supporting concern.

Exposure Limits

Both the US EPA and the European Commission Scientific Committee on Food have recommended a tolerable daily intake of BPA of 50 ppb of bodyweight/day. A report by Korean FDA scientists suggested the maternally tolerable daily intake of BPA should be controlled below 0.127 mg/kg/day.131

Occupational limits for BPA exposure have been set at 5 mg/cubic meter in the US, Germany, and the Netherlands.

At the moment, various governments seem to be taking a wait and see approach around the possibility of low-dose BPA effects. Several governments and related agencies have evaluated many of these low-dose studies, but no radical shift in BPA regulation has been mandated.

US

NIH NTP Low Dose Panel

In August 2001, this group wrote, "There is credible evidence that low doses of BPA can cause effects on specific endpoints. However, due to the inability of other credible studies in several different laboratories to observe low dose effects of BPA, and the consistency of these negative studies, the Subpanel is not persuaded that a low dose effect of BPA has been conclusively established as a general or reproducible finding. In addition, for those studies in which low dose effects have been observed, the mechanism(s) is uncertain (i.e., hormone related or otherwise) and the biological relevance is unclear."132

Dr Pete Myers wrote about this study on http://www.ourstolenfuture.org, "Additional evidence has emerged since the panel met suggesting (1) contaminated food supplies may have led some studies to find no impact; and (2) new independent corroboration of bisphenol A impacts at low levels"

EPA

For BPA, (CAS Number 80-05-7) the EPA has reported a LOAEL for a 1000-ppm diet with an effective dose of 50,000 ppb bodyweight/day.

On March 26, 2002 the EPA issued a document referring to the low-dose phenomena as a "hypothesis," It concluded, "it would be premature to require routine testing of substances for low-dose effects."

FDA

As reported in the in the May 20, 1999 issue of the E/E Letter:

George Pauli, director of the division of product policy at the US Food and Drug Administration (FDA) said the agency is following the low dose issue closely and has seen no reason to take any actions. Addressing the bisphenol A issue, he said "it is troubling that people who appear in good faith to replicate [the vom Saal study] haven't been able to replicate those findings. When you have larger studies intended to replicate a smaller study, and when you do not see the effects, it certainly casts doubt on relying on one study and ignoring the larger ones," Pauli said.

Pauli said that FDA cannot take actions based on vom Saal's research until it has been replicated. "Until you can replicate something, you can't interpret its significance," he said.

Pauli added that FDA has "been working on the chemistry issues, trying to find out if significant levels [of bisphenol A] are really getting into foods, so if anything settles out in the biology area, we'll have a good idea of what humans are exposed to." He reported "with baby bottles, we haven't been able to detect bisphenol A if we use reasonable extraction techniques."

Pauli said that bisphenol A leaches from polycarbonate baby bottles only under exaggerated conditions. "If you heat a bottle with heat and liquid

long enough, you can reverse the polymerization to a certain extent [causing bisphenol A to migrate].”” He said that the testing conditions used by Consumers Union (see E/E Letter Vol. 5, No. 8 & 9), which included boiling bottles for 30 minutes, are not realistic. FDA officials “looked at several baby bottles on the market, and we haven’t seen any directions to boil formula in them,” Pauli said.

“Our conclusion is we should go with the track record. We have evaluated [food contact uses of bisphenol A] in a thorough manner, and concluded its use is safe. We haven’t seen anything that would persuade us to change that.”

European Union

An independent review of the EU Risk Assessment conclusion by the CSTEE (Scientific Committee for Toxicity, Ecotoxicity, and the Environment, an advisory committee to the European Commission), stated, “[A] number of studies using non-standard protocols have reported effects of bisphenol A administration on development using substantially lower doses than the studies performed according to testing guidelines. The RAR critically describes the many weaknesses (lack of repeatability, problems with experimental design and statistical evaluation, poor reporting) of the low dose studies. The CSTEE agrees with the conclusion of the RAR that there is no convincing evidence that low doses of bisphenol A have effects on developmental parameters in offspring and remarks that effects observed are not adverse.”133

The CSTEE also noted, “a number of high quality studies on the reproductive and developmental effects of bisphenol A are already available and do not support low-dose effects.”

In April 2002, the European Commission’s Scientific Committee on Food concluded food contact products made with bisphenol A (BPA) are, and remain, safe for their intended uses.134

Japan

The Japanese Ministry of Health, Labor and Welfare (MHLW) concluded in a December 2001 report, “no reproducible experimental results have been obtained, and at this point of time, it is doubtful whether we can conclude that there are endocrine disrupting effects in the low dose range.”135

A 2002 METI (Japanese Ministry of Economy, Trade and Industry), study report which stated, “Though it is necessary to collect further information on so-called ‘low dose effects’ represented by BPA from academic point of view, it seems unnecessary to take any specific measure other than the above, considering the view expressed by NTP Low Dose Effect Panel that the low dose effect of BPA at present is a phenomenon observed under considerably limited experimental conditions and it is hardly considered to be the general phenomenon.”136

Paradigm Shift in Toxicology?

The significance of this rising interest and reported low-dose effects of BPA also brings up questions around the toxicological process used to screen all chemicals. If new effects and lower doses of concern are discovered for BPA what does that say about the thousands of other widely used chemicals that have not received the same level of scrutiny. If after all of this testing and analysis BPA is found to be unsafe, what should be done about all of the other chemicals less widely tested?

The key question for scientists is whether or not these effects exist and are relevant to humans. As the Czech Institute of Experimental Medicine’s Buckiova noted, “A key question is - what could be an impact of BPA low dose effects (if any exist) on human health and/or reproduction. To it I cannot say a word. I wonder whether outgoing debate could add something to this point.” She suggested one study that might help resolve the debate around BPA would use DNA microarrays, and pubertal males from sensitive and insensitive stocks to look for effects.

Tohyama said, “For some chemicals or radiation, low-dose effects, characterized as ‘hormesis’ have been noted quite a long time, but I would say that not many researchers did even think about the presence of so-called ‘low-dose effects’ and that the monotonous dose-response relation has considered as a kind of dogma in toxicology.

“I would think that so-called ‘inverted U-shape’ responses found in estrogen or DES treat-

ment by vom Saal group have evoked a concern not only to risk assessors but also toxicologists in terms of a paradigm shift in toxicology. If this unique phenomenon can be often, even not always, seen in dose-response relationship for various chemicals, toxicological test guidelines implemented by international and national bodies have to be fundamentally reconsidered. In addition, the possible mechanism(s) behind has to be clarified accordingly. In my understanding, there is several lines of experimental evidence which shows that very low doses of BPA exerted physiological or toxicological responses, but there seems no clear-cut data on 'inverted U-shape' response."

Chahoud, whose research team is currently doing the statistical analysis on a study looking at the effects of 2 ppb of BPA on male and females in-utero noted, "In the last decade, increased attention has been devoted to the identification of Endocrine Active Compounds in consumer products and the environment and their possible impact on the health of humans and wildlife.

"The NOAEL (no observed adverse effect level) dose is an important parameter in the evaluation and risk assessment of chemicals. Along with a safety factor, it is used to determine the daily acceptable intake for humans. Use of the NOAEL is based on the assumption that the dose response curve of a particular substance is linear allowing one to postulate that no effects occur below it. In accordance, attention of toxicological research and risk assessment has been focused on exposure levels above the NOAEL and those beneath it had remained practically ignored.

"Currently, publications regarding endocrine active compounds conducted with doses below the NOAEL have raised serious concerns among the scientific community and the public whether use of the NOAEL in the assessment of compounds with hormone-like actions is appropriate. Analyses of these studies support the notion that possible effects occur in the low dose region. A low dose is generally defined as one below the NOAEL or one, which approximates human exposure. These studies have also indicated that the dose-response relationships can take on variable shapes, such as U-shaped, J-shaped or biphasic ones. Use of a NOAEL dose to calculate acceptable human exposure levels is inappropriate for substances exhibiting these types of dose response curves and, therefore, knowledge regarding the effects in the low dose region is of enormous significance for the evaluation of possible risks for humans and the environment.

"Substances can exhibit an hormetic dose-response relationship, i.e., one in which low doses result in a stimulatory effect and high doses lead to an inhibitory one. This is especially true for hormone-like substances. It is known that the development of reproductive organs before and after birth is under hormonal regulation. This regulation is time- and concentration-dependent. Every variance in terms of time point and concentration of exposure of the regulating hormones can lead to serious abnormalities in development which could manifest themselves in an irreversible fashion at later time points in life.

"All of these facts indicate the necessity for discussion regarding the suitability of the guidelines which exist for the determination, evaluation and risk assessment of endocrine active compounds and, therefore, I think the "excitement" reflects a potential shift in how toxicology is performed and the ramifications this may have on procedures for risk assessment. We have not arrived at the point where we can make general statements for modification of risk assessment procedures and, therefore, a lot more additional studies are required."

Dr. Francesco Dessì-Fulgheri Chair of Ethology at the Dipartimento di Biologia Animale e Genetica, Università di Firenze noted, "The use of very low doses does not reflect the way toxicologists work, but the way behavioral endocrinologist face the problem of brain early development." He said that based on based on the ongoing BPA research the concerns around BPA are still valid, noting, "There is an early action of very low doses on behavior." Dessì-Fulgheri believes the most important endpoints to look at regarding BPA are sexually dimorphic behaviors.

On the other hand, some researchers like Dr. Makoto Ema, with the National Institute of Health Sciences Biological Safety Research Center Division of Risk Assessment, believe "this excitement is more

specific to BPA" rather than about the paradigm used to assess chemicals.

Syngenta's Ashby said, "Irrespective of whether low doses of BPA do, or do not, produce effects in rodents, two higher issues have been raised by these failures to confirm initial observations. First, in such a complex area, investigators should take the time to conduct repeat experiments - a basic rule of good science, in any case. Second, assuming that BPA does produce effects, we must seek an explanation for why genuine attempts to confirm these effects can be unsuccessful. Until the latter point is resolved no progress can be made in routine screening for the assumed effects"

Conclusion

This survey raised more questions than answers. It is true that researchers are finding lots of different effects at environmentally relevant doses. But to our knowledge, no experiments suggesting low-dose effects have ever been faithfully replicated across labs. Then there are questions about the meaning of factors like prostate size or behavior. Should a chemical be more tightly regulate because it causes changes in behavior? If so should television be next?

On the other hand, the Wetherill et al (2002)111 results suggesting prostate changes that could interfere with cancer treatment are interesting, considering the prevalence of this disease.

And if based on the evidence, regulators eventually decide to do something about BPA, where do they start and stop? Baby bottles, baby food cans, and medical devices seem like an obvious place to start because of the higher potential susceptibility. But then what would it be replaced with? Glass bottles break and are heavy. If there was a switch how many kids would get cut up by broken bottles compared to those saved from hormonal disruption?

There are also questions about chemical alternatives. If a widely used industrial chemical like BPA is found to have profound problems 20 years after it was declared safe at environmental levels, how are we going to choose safer alternatives?

Sidebar 1: Why Bisphenol-A is Attractive

Polycarbonates generally cost more than other plastics because they don't break easily; have a higher melting point and can be made in a variety of colors.

CDs and DVDs

BPA's high heat resistance, excellent flowability and optical characteristics have attracted CD makers. Over 110 billion shiny BPA disks have been produced since their debut in 1982, with about 25 billion produced in 2001 alone, according to Bayer statistics. Bayer expects the demand for polycarbonate in optical media to reach 800,000 metric tons for the 44 billion disks anticipated in 2005.

Once the BPA has been converted into plastic, it becomes relatively stable. The main concern with the sheer number of CDs has less to do with toxicology than land fill. While 25 billion CDs make their way into the world, only a tiny fraction are recycled. Only about 2,000 metric tons of polycarbonate become available for recycling every year as rejects, leaving the other 99% of the material to find its way into landfills and interesting artistic shiny disk installations.

Glass Replacement

BPA is attractive as an alternative to glass in applications like car windshields and greenhouses. However, it scratches easily, so manufacturers are developing coatings to protect the surface. Proponents of this approach believe that as much as 75 pounds in weight could be saved by the substitution of plastic for glass in the average 4-door sedan. (http://www.usglassmag.com/backissues/9901/9901k98plastics.html)

Gummy Resin in Metal Cans

The number of metal food cans (31 billion in 1972 to 24 billion in 1999) has been dropping while beverage uses (33 billion in 1972 to 102 in 1998) are on the rise, according to the Can Institute

Other applications

Films, sheets, and laminations
Reinforced pipes floorings
Watermain filters
Enamels and vanishes adhesives
Artificial teeth
Nail polish
Electric insulators
Epoxy resins

Food and Oral Uses of BPA

Plastic coating for children's teeth to prevent cavities

Coating in metal cans to prevent the metal from contact with food contents

Plastic in food containers

Refrigerator shelving

Baby bottles

Returnable containers for juice, milk and water,

Microwave ovenware

Eating utensils

Sidebar 2: Who's Who in BPA Biz:

General Electric (Lexan) and Bayer (Makrolon), and Dow (CALIBRE) are the leading BPA producers.

Sidebar 3: BPA Resources on the Web

Informational Sites

Polycarbonate overview: http://www.apme.org/polycarbonate/
Resins in Cans Overview: http://www.cancentral.com/
Epoxy Resin Systems Task Group: http://www.socplas.org/about/epoxy/
Niosh: http://www.cdc.gov/niosh/rtecs/sl602160.html
SRI Consulting: http://ceh.sric.sri.com/Public/Reports/619.5000/

Highlighting safety of BPA

Bisphenol-A Site: http://www.bisphenol-a.org/
ADA on Bis-A: http://www.ada.org/prof/prac/issues/statements/seal-est.html
NTP Report 2001: http://ntp-server.niehs.nih.gov/htdocs/liason/LowDoseWebPage.html

Highlighting concerns about BPA

Our Stolen Future: Http://www.ourstolenfuture.org/NewScience/oncompounds/bisphenola/bpauses.htm
Warhurst: http://website.lineone.net/~mwarhurst/bisphenol.html
WWF Report on Bis-A: http://www.wwf.org.uk/news/n_0000000145.asp
BPA in baby bottles: http://www.mindfully.org/Plastic/Plasticizers/BPA-Baby-Bottle2apr02.htm
National Environmental Trust on Baby bottles: http://environet.policy.net/health/products/plasticwrap.vtml

Sidebar 4: Comments about the draft of this survey

Steve Hentges, with the American Plastics Council said, "For what is intended to be a survey, presumably thorough and balanced, the draft is surprisingly one-sided. Studies that claim effects are extensively catalogued and described, yet studies that cannot replicate the same effects are only mentioned in passing, even though the attempts to replicate are invariably larger and more comprehensive studies. Same for the comprehensive multi-generation studies, which are only briefly mentioned in spite of the significance and weight applied to these studies for safety assessments by governmental bodies worldwide. Overall, the draft comes across as a biased view."

"The section on human exposure mentions reported measurements of BPA in blood but ignores papers that both report BPA (as the glucuronide metabolite) in urine and the daily dose estimates directly derived from these measurements. For example, I've attached a paper that summarizes some of these results, but there are other more recent ones that may be even better (See JSEDR BPA Assessment). These papers show that actual

human exposure to BPA is extremely low.

"The human/animal comparison section is missing a key paper on the kinetics/metabolism of BPA in humans (Volkel et al, 2002)137, which describes a study on human volunteers.

"The section on Questions About Effects starts off with a comparison to tobacco research. There seems to be little relevance of this WWF quote to your survey."

Barbara Elswick, a CIIT researcher wrote, "My one concern is the emphasis on the polycarbonate cages. I don't believe they are likely to be an issue with rodents. Rodents don't lick cages and there is bedding to soak up any water drips, so I don't see how they would get exposed.

"Our abstracts weren't included which is fine as I'm close to having manuscripts ready to submit for publication. However, how about the Kwon138 negative study which was out of the Welsch lab?"

Dr. Ibrahim Chahoud with the Institute of Clinical Pharmacology and Toxicology in Germany wrote, "Congratulations for the excellent survey on BPA. I read the article today and I agree with it. Thank you."

Footnote

Part of the confusion in assessing the risks of BPA lie in the different ways of quantifying doses. Researchers report on ug/kg, mg/kg, parts per million (ppm) and parts per billion (ppb). Furthermore these numbers can reflect either the absolute concentration of BPA in a sample or as a daily dose that reflects the amount of BPA in relation to the recipient's body weight. In order to simplify this aspect of the BPA debate we are listing all BPA concentrations in ppb or ppb/bodyweight/d (parts per billion/bodyweight/day). It should also be noted there are substantial difference in the amount of BPA available to the recipient when given orally, compared to via s.c. injection. Some of the reviewers of the draft felt that this non-standard approach was confusing because it has not been used before.

Steve Hentges with the American Plastics Council said, of this approach "The non-standard units you've used (e.g., ppb of bodyweight/day) are confusing. I don't think I've ever seen anyone use units like this."

Our current view is that choosing one representation of measurement, and sticking to it provides a better way of comparing exposure and the relative level at which effects are reported.

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