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COMMENTS ON NTP APRIL 2008 DRAFT REPORT ON BISPHENOL A (BPA)

These comments are offered in the order in which the points they address appear in the April 2008 draft.

Comment #1. Typographical Error in Figure Legend.

Figure 1. Correct the typographical error: The molecular weight of BPA is 228.28.

Comment #2. Effect of Age and Heat on Bisphenol A Migration from Polycarbonate.

P 4. "The degree to which bisphenol A migrates from polycarbonate containers into liquid appears to depend more on the temperature of the liquid than the age of the container, i.e., more migration with higher temperatures (*4*)"

The Le et al (Ref 4) paper did not systematically examine age of the products, they just asked people to give the previously purchased bottles. The conclusion about "age" is inconsistent with data from experiments we have conducted and will soon publish that repeated washing increases the rate of leaching of BPA from sport bottles and baby bottles into water at room temperature, although the statement about increased heat accelerating leaching is consistent with every publication. The statement about "age and leaching" needs to be changed to say that age and repeated washing accelerate leaching, as does increased heat, acidic or basic substances. This is also able to be determined from any introductory text in organic chemistry, which covers the conditions under which hydrolysis of ester bonds (that link BPA molecules in polycarbonate) occurs. The best references for repeated washing and "age" increasing leaching, independent of heat, are: (Brede et al. 2003; Howdeshell et al. 2003).

Comment #3. Issue of Sources of Exposure and Method of Calculating Exposures.

P 4. "Scientists can also add, or aggregate, the amounts of bisphenol A detected in various sources, i.e., food and beverage, air, water, dust. The approach of aggregating exposure to estimate daily intake requires sources of exposure to be

known and measured. In general, estimates based on biomonitoring are preferred for calculating total intake because all sources of exposure are integrated into the fluid or tissue measurement and do not have to be identified in advance. Estimates based on sources of exposure are useful to help discern the relative contributions of various exposure pathways to total intake."

P 5. "Following ingestion, the majority of bisphenol A is quickly bound to glucuronic acid to produce bisphenol A-glucuronide, a metabolic process called glucuronidation that is carried out by enzymes primarily in the liver [reviewed in (*2*)]."

The process used to "back calculate" BPA exposure described on P 32 in this brief assumes very rapid and virtually complete metabolism after oral exposure to BPA, as stated in the quote above. As reviewed by Vandenberg *et al*. 2007, this assumption is not consistent with data from numerous experiments measuring unconjugated BPA in human blood using a variety of analytical methods. The draft NTP brief rejects studies that used ELISA to measure BPA in blood. But there are multiple non-ELISA based studies that report on unconjugated BPA levels in human blood that are ignored.

The rejection of studies that measured BPA in blood by ELISA does not warrant the assumption that there is no BPA found in the blood of humans, which is the basis for the "back calculation" procedure to estimate exposure of adult humans based only on total BPA levels (conjugated and unconjugated) in urine.

Schonfelder et al 2002 (Ref 239) is just one study contradicting this method of calculating adult human exposure. An excellent review by CDC scientists of the criteria that should be used to identify a rapidly metabolized chemical is (Barr et al. 2005). By their criteria, the published data on blood levels of unconjugated BPA in humans indicates that BPA is not rapidly and completely metabolized in adults after oral exposure. Hence it is wrong to conclude that it is.

Since 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 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 routes of exposure other than the oral exposures from food and beverage containers considered in the NTP brief. At this time, there is no requirement anywhere in the world to identify the chemicals used in any product, such as baby toys, food and beverage containers or paper products. The issue of BPA in paper products is interesting in that printer's ink contains BPA, and the "carbonless paper" used to provide receipts for purchases is coated with BPA. Newspapers, carbonless paper and other paper products could be significant sources of trans-dermal BPA exposure and also inhaled exposure (the BPA is coated on carbonless paper as a dust at parts-per-thousand level). Because these paper products are often recycled, most if not all recycled paper has significant BPA contamination. Dermal absorption of BPA from these potential sources of exposure has not been investigated. In addition, BPA is a known additive in PVC plastic products, such as PVC dolls and toys and the myriad PVC household products and building materials. How much BPA detected by biomonitoring comes from PVC remains uninvestigated.

These potential sources of non-oral exposure are ignored in the calculation of adult exposure to BPA in the NTP draft and could be why blood levels of BPA are much higher than the "rapid metabolism" model predicts. When published data contradict assumptions, the assumptions should be changed!

It is clear that fetuses lack the liver enzymes that conjugate BPA and newborns have limited capacity to conjugate BPA. As stated in the brief, the above issue of "rapid and complete metabolism of orally ingested BPA" does not apply to the analysis of exposure of infants, which are recognized to likely have the highest blood levels of biologically active BPA, although this has not been examined in humans. In rodents, there are published studies that show a marked decrease (about 10-fold) in bioactive BPA (as well as the drug diethylstilbestrol or DES, which is conjugated by the same enzyme) between birth and weaning (Fischer and Weissinger 1972; Matsumoto et al. 2002). We reported that route of exposure (subcutaneous injection vs. oral administration) has **no** impact on unconjugated BPA in serum during the 24 hours after administration in newborn mice (Taylor et al. 2008), consistent with the above findings that newborn rodents have low levels of the conjugating enzymes required to metabolize BPA. This has also been known for human infants for decades for this class of enzymes, although not specifically studied with regard to BPA.

What was not mentioned in the brief is that Matsumoto and colleagues found in rats that during pregnancy, there was a significant decrease in maternal glucuronidation of BPA and other chemicals relative to non-pregnant adult females; they state "rat hepatic microsomal UDP-glucuronosyltransferase activities toward these xenoestrogens were reduced by about half during pregnancy of mother rats" (Matsumoto et al. 2002). This has important implications for exposure of fetuses, since significantly more BPA may escape first pass conjugation in pregnant women, thus accounting for the finding by Schonfelder et al. 2002 (Schonfelder et al. 2002) (Ref 239) that levels of unconjugated BPA in the blood of pregnant women in Germany have virtually the same median, mean and range of urine total BPA reported by the CDC (Calafat et al. 2008) (Ref 6). This issue should be identified as important in the NTP brief and argues that with specific regard to adult pregnant women, the assumption of "rapid and complete first pass metabolism of BPA" is not consistent with the published data from animal studies.

A final comment is that in determining which studies to use in determining human exposures or rates of metabolism, close attention needs to be paid to the limit of detection of the assay. A number of studies that were relied on in the NTP brief had high limits of detection relative to what would be expected in the 21st century. The section on human metabolism of BPA needs to be re-examined with this in mind. This will be greatly aided by using the table in the Vandenberg et al. 2007 article (Ref 3) that reports the limit of detection in each publication. Studies using insensitive methods that conclude that BPA is rapidly metabolized because it cannot be detected should be interpreted with caution, particularly if the authors are associated with corporations with a financial interest in this issue.

One study that is cited a number of times in the draft report is by Domoradzki (Domoradzki et al. 2004) (Ref 12). This industry-funded study dramatically "dummed down" the assay sensitivity in order to arrive at a pre-determined conclusion. This study used methods approximately 10,000-times less sensitive than the methods we used in a related study

(Taylor et al. 2008) that contradicted the conclusion by Domoradzzki that low doses of BPA could not affect neonatal rodents, the pre-determined conclusion that the study was designed to find. They acknowledge that "Half-life determinations were based on pooled plasma samples through 24 h and, therefore, these estimates may not be reliable", and they only used 3 animals per group. Most important, however, is that the article contained no statistical analyses at all, yet the authors drew comparisons between groups that suggested that statistically significant effects had been observed. This study is not worthy of being cited anywhere, particularly in a NTP report. The finding that there is an increase in the rate of conjugation of BPA and other chemicals between birth and weaning (reported by Domoradzki) was also reported by Matsumoto et al. (Matsumoto et al. 2002), and this article should be cited as evidence for this issue.

Comment #4: Issue of Level of Concern for Low Dose Developmental Exposure in Laboratory Animal Studies.

P 9. The report states: "When considered together, the results of "low" dose studies of bisphenol A provide *limited evidence* for adverse effects on development in laboratory animals (see Figures 2a & 2b)."

The published peer-reviewed literature does not support this low level of concern and suggests that, similar to the FDA, the NTP is being unduly influenced by a relatively small number of published findings of "no harm" due to 100% of industry-funded studies reporting no harm in response to low doses of BPA, while most of the remainder of the "no harm" low dose findings are due to the use of animal models that are insensitive to any exogenous estrogen. This contrasts with harm being reported in over 90% of government-funded studies with low doses of BPA conducted by government and academic scientists with a very high level of skill in the area being studied.

Evidence of adverse effects due to exposure to low-doses of BPA during development are found for the female mouse reproductive organs (Newbold et al. 2007), alignment of chrosomes in fetal mouse oocytes (Susiarjo et al. 2007), neuroendocrine function in rats (Rubin et al. 2001) and mammary gland structure and gene activity in rats and mice (Markey et al. 2001; Nikaido et al. 2004; Vandenberg et al. 2006; Durando et al. 2007; Vandenberg et al. 2007). For most endpoints examined in these studies, there are no contradictory findings from studies that reported no harm in response to low doses of BPA, because they did not examine the same endpoints.

There is a large literature on neuro-anatomical changes, neuro-chemical changes and behavioral changes due to low dose exposure to BPA during development (reviewed in: (Richter et al. 2007). No study reporting no harm in response to low doses of BPA has examined any of these outcomes, and there are thus no finding contradicting these results. Studies on the developing male reproductive system are discussed in detail below. Taken together, the existing peer-reviewed literature on low-dose effects of BPA due to developmental exposure does not warrant the conclusion of "limited evidence for adverse effects in laboratory animals".

An example of how the conclusion of “minimal concern” results from not connecting the data from related experiments is revealed by examining our findings about the effects of estrogen: endogenous estradiol as well as the estrogenic drugs DES and ethinylestradiol, and BPA in a series of articles based on research conducted at the University of Missouri, (a number of these studies were conducted in collaboration with scientists at other universities) beginning in 1992 through 2007 (Nonneman et al. 1992; Nagel et al. 1997; vom Saal et al. 1997; Timms et al. 1999; Timms et al. 2002; Timms et al. 2005; vom Saal et al. 2005; Richter et al. 2007).

A major finding is that estrogenic chemicals such as BPA and DES stimulate prostate development at very low doses and inhibit prostate development at high doses. These conclusions are now supported by multiple studies independent of industry funding (See below why industry-funded attempted replications have failed).

This observation started with the finding that male mice located *in utero* between female fetuses (2F males) have higher levels of serum estradiol relative to male fetuses that develop between male siblings (2M males) (vom Saal 1989), and male mice with elevated fetal estradiol have enlarged prostates in adulthood and elevated numbers of androgen receptors. We then examined only male mice that developed between a male and a female fetus (1MF males) to reduce background variability in serum estradiol; in this experiment we implanted pregnant mice with Silastic capsules containing increasing doses of estradiol. At a maternal dose that increased free (unconjugated and unbound to plasma estrogen-binding proteins) serum estradiol by 0.1 parts per trillion, the number of prostate ducts and size of prostate ducts was increased in male CF-1 mouse fetuses (based on computer-assisted reconstruction), and in adulthood, prostate size was increased and the number of prostatic androgen receptors was increased. An interesting related finding was that the volume of the urethral canal was reduced.

Very importantly, high levels of estradiol did not stimulate an increase in prostate size, and the same inverted U dose-response relationship was observed for the effect of orally administered DES to pregnant mice, with prostate size being significantly stimulated by 0.02 µg/kg/day and inhibited at 200 µg/kg/day. Subsequently, we found that at the maximum response dose of 0.1 µg/kg/day of DES and ethinylestradiol (administered orally to CD-1 pregnant female mice), and a dose of 10 µg/kg/day BPA, relative to controls, there was an increase in prostate gland number in male 1MF mouse fetuses, an increase in prostate duct size, and an increase in proliferation of basal (stem) cells in the primary prostatic ducts. This finding suggested that since basal cells are implicated in the development of prostate cancer, BPA and other estrogenic chemicals and drugs might cause prostate cancer (Timms et al. 2005).

This result was confirmed by Ho, Prins and colleagues in rats where a dose of 10 µg/kg/day to neonates resulted in early stage prostate cancer in adulthood (Ho et al. 2006). We also observed malformations of the urethra caused by BPA and the estrogenic drugs. Subsequently, we showed that in primary culture of fetal CD-1 mouse prostatic mesenchyme, addition of 0.28 parts per trillion estradiol or 0.23 parts per billion BPA significantly increased prostatic androgen receptor and estrogen receptor alpha (ESR1) gene activity, as predicted by the in vivo experiments.

A permanent increase in the production of androgen receptors in the prostate significantly increases the risk for prostate disease. Hper-sensitivity to hormonal stimulation in any hormonally regulated organ that is prone to disease would be predicted to increase the risk of disease.

Many critics have ignored the consistent finding of an increase in androgen receptors by estradiol, DES and BPA (Nonneman et al. 1992; vom Saal et al. 1997; Gupta 2000a; Richter et al. 2007) and, instead, concluded that an increase in prostate size was of no consequence for risk assessment purposes. This is clearly not a rational scientific conclusion given that prostate size increase is related to hyper-responsiveness of the prostate to androgen due to a permanent increase in androgen receptors. The recent findings by Richter and colleagues (Richter et al. 2007) suggest that there may also be a permanent increase in estrogen receptors caused by fetal exposure to estrogenic chemicals such as BPA, but this has not been examined.

Our prostate findings were replicated by an independent academic scientist who directly examined the effects of BPA and DES on prostate development in mice, using a combination of *in vivo* and primary culture studies in one report (Gupta 2000a) and primary culture in another (Gupta 2000b). This investigator had spent her entire career publishing articles on the effects of hormones on development of the rodent reproductive system, and her study (Gupta 2000a) directly replicated our findings and then showed that in primary culture of the prostate collected from CD-1 mouse fetuses, a 0.1 parts per trillion dose of DES (her positive control) and 50 parts per trillion of BPA directly stimulated prostate gland development. In vivo, an oral DES dose of 0.1 µg/kg/day or BPA at 50 µg/kg/day increased prostate size and prostatic androgen receptors.

Two chemical industry trade organization-funded studies were quickly conducted and published (Ashby et al. 1999; Cagen et al. 1999) after our initial findings were published in 1997 (Nagel et al. 1997; vom Saal et al. 1997). Both of these studies were conducted using Good Laboratory Practices (GLP). The conclusion from both of these studies was that there was no effect of low doses of either BPA or DES (the positive control used in both studies) on prostate development. The chemical industry has used these “negative” findings to create doubt about the validity and reliability of our findings and those of Gupta.

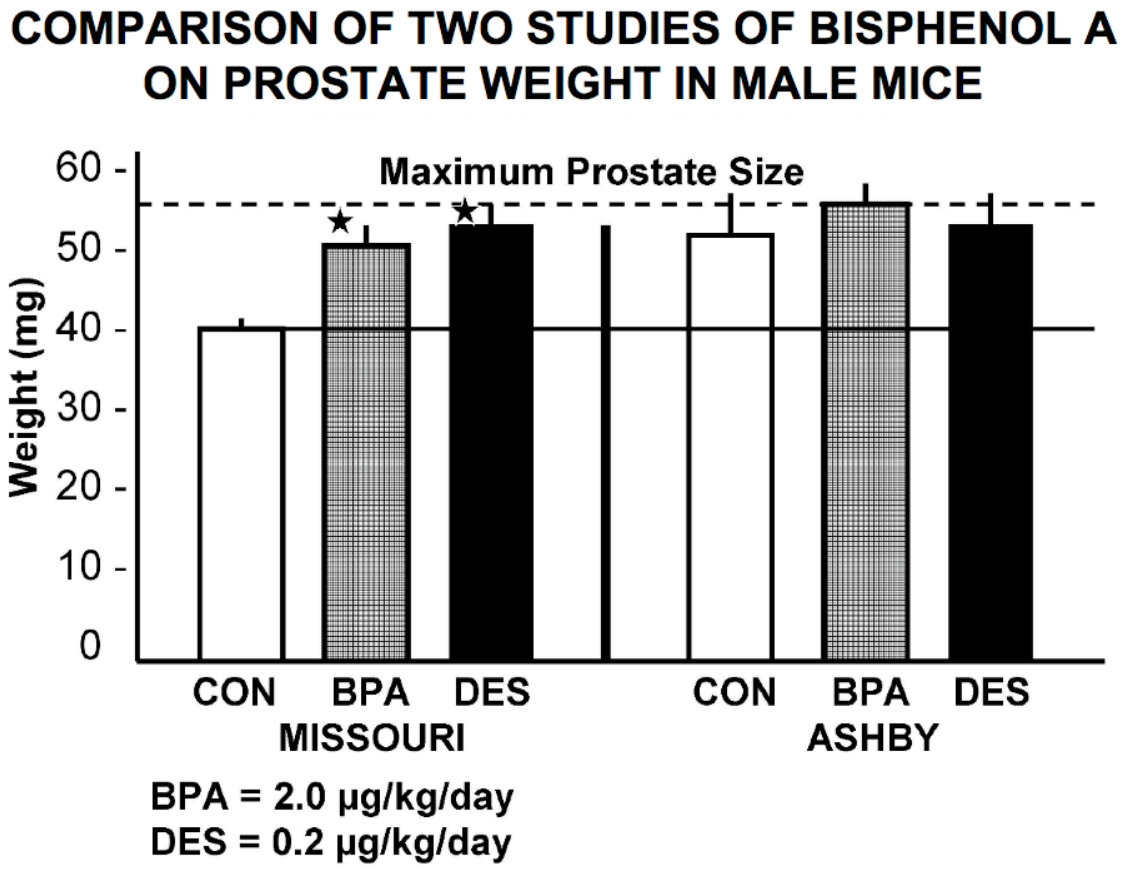
A brief comment about GLP is needed here. GLP was instituted due to finding of repeated incidents of fraud by commercial labs contracted to test chemicals by industry. GLP does not mean the study was done correctly or by competent people, only that it was done and that extensive paperwork is available documenting all details of the experiments. GLP experiments are conducted only by industry due to the paper work and high expense required. For academics, this issue of the reliability of results is addressed by independent replication, such as the replication of our findings by Dr. Gupta, which led to an editorial stating that this replication essentially ended the argument about whether BPA could alter prostate development in mice (Sheehan 2000).

The question is, **why did industry-funded efforts fail to replicate our studies**, when academic experiments successful replicated them and when subsequent studies examined the molecular mechanisms and computer-reconstructions of microscopic growth patterns in much greater detail? These academic studies not only confirmed our original results, but

extended them with new analytical tools. Industry-funded studies, in contrast, continued to focus, unsuccessfully, on questions that were appropriate for the 1990s, but that were supplanted by advances in developmental endocrinology.

We have no way to determine what accounts for the lack of positive findings in the Cagen study (Cagen et al. 1999). No one has ever been able to access the Cagen *et al* data and find out why the initial cohort of animals that were produced at MPI Laboratories (where the experiment was conducted) were suddenly killed, and the experiment was started again and then terminated 3 months earlier than called for in the initial protocol. However, I have published a detailed review of the Ashby failure to replicate (vom Saal and Welshons 2006), and the conclusion (supported by Figure 1 below from this publication) is that the only difference between our University of Missouri study and the study conducted by Ashby was that his negative control values were at the maximum for the CF-1 mouse, as shown in our prior studies revealing an inverted U dose-response curve for fetal estrogen exposure and adult prostate size in CF-1 mice (vom Saal et al. 1997).

FIGURE 1 (from: (vom Saal and Welshons 2006).



The fact that neither Ashby nor Cagen acknowledged in their published articles that their studies had been designed with DES as a positive control, and that the positive control had failed to show a significant difference from the negative control, is an example of fraudulent misrepresentation of results in that it is unacceptable to misrepresent the design of an experiment based on the results not turning out the way the researchers wanted. This had

the desired effect on the FDA, however, as the FDA official in charge of monitoring BPA stated in an interview:

> May 20, 1999
> The following was reported in the Endocrine/Estrogen letter Vol. 5, No. 10 (106), published by Global Press.
> **FDA Reaffirms The Safety Of Plastic Baby Bottles**
> 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 that "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.

I return here to the issue regarding the fact that GLP is constantly invoked as meaning that a study was conducted using higher standards than those used in academic or government laboratories, and that GLP study findings should be accepted as valid and reliable, while academic studies using smaller numbers of animals should be disregarded if an industry-funded GLP study contradicts the findings. First, not one investigator on either the Ashby or Cagen publications (Ashby et al. 1999; Cagen et al. 1999) had ever been associated with a publication concerning the study of the male reproductive system, and no one associated with these studies had any idea how to conduct the study. This stands in direct contrast to the requirement for funding by NIH or other government agencies that provide funds to academic scientists that they provide preliminary data that would allow reviewers to identify “demonstrated competence”. So, how did these two groups proceed? Each of these groups asked me to train them in how to conduct the research, which they though could be accomplished in a few hours! This exemplifies the level of expertise that general contract labs believe is necessary to conduct toxicological studies. In sharp contrast, the research in my laboratory, and the research conducted in other academic laboratories, involves people at the Ph.D. level with an extremely high level of skill.

The CERHR BPA panel reached the same conclusion about the GLP Ashby and Cagen CF-1 mouse studies: “This paper is inadequate for the evaluation process due to absence of response of the positive control group.” The Ashby study was also criticized for using a small number of animals, although you would never know that based on the statements made above about the study by the FDA official.

As a result of the criticism of the two coordinated studies conducted by Ashby and by Cagen (that were supposed to use the same design, but ended up with unexplained differences) in which the positive control failed to show an effect, the plastic industry funded the Research Triangle Institute (RTI) to conduct a study that did not include a positive control group. However, the RTI experiment, which involved over 8,000 rats and cost over one-million dollars, used a rat (the CD-SD rat) that had been subjected to selective breeding for over 50 years by the breeder, Charles River Laboratories. The consequence of this selective

breeding for large litter size and a rapid rate of growth is that these rats have become *insensitive* to treatment with any estrogen, including the estrogen used in birth control pills.

Background: A low-dose birth control pill contains 20 µg of the estrogenic drug ethinylestradiol (there are also pills with 30 µg of ethinylestradiol), and the average woman in the USA weighs about 70 kg according to the CDC. The average dose of ethinylestradiol in a birth control pill is thus between 0.3 and 0.4 µg/kg/day ethinylestradiol, and a typical woman taking these pills for one month (30 days) would receive a total of between 9 – 12 µg/kg ethinylestradiol.

Yamasaki *et al.* (2002) reported that the CD-SD strain of rat showed some responses to 50-µg/kg/day ethinylestradiol administered for 28 days, and more responses to the very high dose of 200 µg/kg/day (Yamasaki et al. 2002). The CD-SD rat thus requires a dose of ethinylestradiol at least 25,000-times greater than the dose that alters prostate and testis development in male mice, which respond to an oral dose of 0.002 µg/kg/day administered to pregnant females (Thayer et al. 2001). Of great importance is that 100% of the 13 studies conducted with the CD-SD rat conclude that low doses of BPA cause no harm (this information is posted on my web site: http://endocrinedisruptors.missouri.edu/vomsaal/vomsaal.html, and has also been published in a peer-reviewed article (vom Saal and Hughes 2005). Studies with this rat account for 45% of the studies that report "no harm" in response to low doses of BPA.

A recent study used another rat (the Long-Evans or LE rat), and reported that this rat required between 5 – 50 µg/kg/day of ethinylestradiol to show responses that were also examined in animals exposed during development to low doses of BPA (Howdeshell et al. 2008). At least for the responses that were examined by these investigators, this rat is thus also insensitive to low doses of any estrogen, including doses of ethinylestradiol used in birth control pills, as shown by the positive control findings reported in the study.

The assay sensitivity as determined by the dose of the positive control predicts whether the experiment is appropriate to reveal low dose effects of BPA. In this case (Howdeshell 2008 #935) the appropriate conclusion, which was not explicitly stated in the discussion, should have been that based on the high dose of the positive control required to elicit the responses being investigated, the LE rat was an inappropriate animal model to use to assess the potential for low doses of BPA to impact humans, since humans are clearly more sensitive to treatment with estrogen than are these rats.

Finally, a study was just published in which CD-1 mice were examined for low-dose effects of BPA, again by RTI Laboratories and funded by the plastic industry (Tyl et al. 2008a). This is important because the CD-1 mouse is the model animal used by the laboratory of reproductive toxicology within the NTP, and this mouse has been documented to respond to low doses of DES and BPA: specifically, there are 21 peer-reviewed studies with the CD-1 mice showing low-dose BPA effects (these are listed at: http://endocrinedisruptors.missouri.edu/vomsaal/vomsaal.html).

In science, this amount of confirmation is accepted as greatly exceeding the criterion that findings of low-dose effects be independently replicated. It was thus surprising to find that the conclusion from the Tyl *et al*. study was that no low-dose effects of BPA were observed.

However, the authors of this study published a companion article (Tyl et al. 2008b) showing that in their laboratory, the CD-1 mouse required a dose of the positive control estrogen (estradiol-17ß) of 100 µg/kg/day, which is an extremely high dose. Thus, whether because of laboratory contamination (perhaps associated with a fire involving BPA-containing rat cages), the type of feed used, Purina 5002, which has been discussed in detail as inappropriate for use in endocrine disruptor research (Heindel and vom Saal 2008), or due to some other reason, this highly estrogen-responsive mouse was unresponsive to low doses of either the positive control or BPA. Once again, the inability of animals to respond to low doses of the positive control estrogen predicted the absence of a finding of harm caused by low doses of BPA.

In the NTP Brief (p 14) it was stated that: "The NTP concurs with the opinion of several scientific panels that positive control groups can be very useful to evaluate the sensitivity and performance of a given experimental model (2, 52, 63). However, the NTP does not consider use of a positive control to be a required study design component particularly in animal model systems that are well characterized regarding the background incidence of "effects" and their variability."

This conclusion contradicts the conclusion reached at two NIH-sponsored workshops on variability in feed as a basis for non-replication in laboratory animal research (Heindel and vom Saal 2008). Because soy-based feed varies in soy phytoestrogens (primarily genistein and daidzein) as well as other estrogenic chemicals not detected by chemical analysis of soy phytoestrogens, such as the mycotoxin zearalenone, the conclusion was that use of historic data are inappropriate and experiments that report no significant effects of a chemical, such as BPA, must include a concurrent positive control (Heindel and vom Saal 2008).

What the NTP Brief failed to identify was that while a positive control effects was considered valuable, the requirement that the positive control show a "low dose" effect was a glaring omission in the report. The absence of a low-dose positive control effect in the Tyl *et al*. study renders invalid the conclusion by Tyl *et al*. (Tyl et al. 2008a) that "BPA is not considered a selective reproductive or developmental toxicant in mice". To ignore this issue is to ignore a profound flaw in Tyl *et al*.'s experiment.

A related issue is that general contract labs such as RTI are not centers of expertise on specific outcomes being studied, and their approaches tend to be very crude compared to the types of outcomes examined in academic and government laboratories staffed by people with a very high level of expertise in the specific outcome that is chosen as the focus of a particular study. These contract laboratories thus do not do computer reconstructions of the fetal prostate coupled with immunohistochemistry on the slides used for the reconstruction, nor do they typically measure gene activity or examine whether epigenetic changes have occurred, as shown by Ho et al. and Dolinoy et al. for BPA (Ho et al. 2006; Dolinoy et al. 2007).

The FDA has continued to ignore the large number of studies published by academic and government scientists showing low-dose effects of BPA, and the FDA recently identified in a letter to the US House of Representatives that their conclusion that BPA was safe was

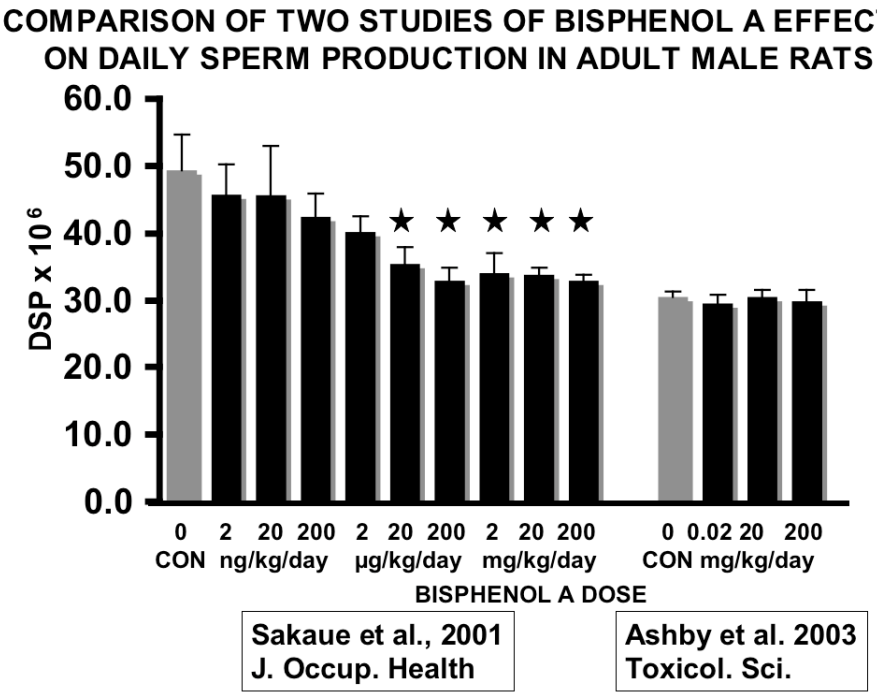
based on only the Tyl *et al* 2002 study (Tyl et al. 2002) and the Tyl *et al*. 2008 study {Tyl, 2008a #(Richter et al. 2007).house.gov/Investigations/Bisphenol.shtml).

Comment #6: Issue of Lack of Concern for Adult Exposure to BPA in Laboratory Animals.

Basically, this conclusion is difficult to understand, unless, as above, undue weight is being placed on studies that did not include a positive control and simply reported that no harm associated with exposure to BPA was observed.

An example of what happens to the argument when the findings of studies reporting harm and those reporting no harm for the same outcome are analyzed in detail is revealed by comparing a study conducted at the Japanese NIH {Sakaue, 2001 #131} and a supposed "exact" replication was attempted in the laboratory of John Ashby at Zeneca (now Syngenta) (Ashby et al. 2003). The results of these studies are presented side by side in Figure 2 and have been reviewed in detail in a prior publication (vom Saal and Welshons 2006). It is clear that, similar to the comparison of the prostate findings from my laboratory and the findings from the Ashby laboratory, once again, the only diffrence between the two studies was that the negative control data from the Ashby study were at the maximum amount of suppression of testicular sperm production observed in the Sakaue study. My interpretation of this finding, when considered in relation to the prior prostate findings, is that the Ashby laboratory is contaminated with estrogenic chemicals that are resulting in a maximum response in the negative controls. In contrast to this obvious conclusion, Ashby stated in his abstract: "No explanation for our failure to replicate the effects reported by Sakaue et al. is evident."

FIGURE 2 (from: (vom Saal and Welshons 2006).



I suggest that the NTP re-evaluate the conclusion that there is no concern with regard to adult exposure to low doses of BPA in laboratory animals. Perhaps this is due to the misguided assumption of very rapid metabolism of BPA in adult animals, which would make concern about exposure meaningless. There are large number of studies showing adverse effects in adult rodents, which has been reviewed by Richter et al. (Richter et al. 2007). These findings include the research conducted in the laboratory of Dr. Nadal showing direct effects of very low doses of BPA on pancreatic ß cell insulin secretion in mice followed by insulin resistance. Such findings should not be dismissed, particularly since the molecular pathways mediating the responses are being discovered (Alonso-Magdalena et al. 2005; Alonso-Magdalena et al. 2006; Alonso-Magdalena et al. 2008).

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