Recent Evidence for Low Dose Effects of Bisphenol A

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Bisphenol A has become one of the primary endocrine disrupting chemicals being chosen for study by researchers in many different disciplines who have recently become aware of the issue of endocrine disruption. These articles are being published in a wide range of journals outside of the field of toxicology, and new findings are appearing every month.

"Low dose" refers to doses that are lower than those typically used in toxicological studies, and this also refers to doses that are within the range of human exposure.

For risk assessment purposes, the Society of the Plastics Industry has recommended using 50,000 μ g/kg/day as the no effect dose (NOEL). Doses below 50,000 μ g/kg/day would thus be considered to fall within the "low dose" range.

In this talk the following topics will be discussed:
1. Endogenous hormones cause effects at very low doses
2. Low dose effects of bisphenol A in males and females
   and potency of bisphenol A relative to DES
3. Potential mechanisms of low dose effects of bisphenol A
4. Components of animal feed are endocrine disruptors and must be considered
   when conducting endocrine disruptor studies
5. Implications of inverted-U dose-response curves for risk assessment

The "low dose" issue in toxicology was shown to be important by studies that demonstrated that endogenous hormones, such as estradiol, caused permanent effects in fetal tissues at extremely low doses. For example, an increase in free (bioactive) serum estradiol of 0.1 pg/ml (0.1 ppt) in CF-1 mouse fetuses (due to maternal treatment) resulted in a permanent increase in prostate size and prostate androgen receptors. When we found that bisphenol A did not bind to plasma estrogen binding proteins which protect fetuses from exposure to high levels of estrogen present during pregnancy, the low dose studies with estradiol and other estrogenic chemicals, such as DES led to studies of low doses of bisphenol A.

Gupta reported that in CD-1 mice that administration of bisphenol A to pregnant mice at a dose of 50 μ g/kg/day resulted in a permanent increase in prostate size and androgen receptors (bisphenol A also caused a decrease in the size of the epididymis). These findings were virtually identical to those I reported for estradiol. Gupta's findings also replicated the findings concerning bisphenol A from my lab. We administered 2 and 20 μ g/kg/day bisphenol to pregnant mice and reported permanent enlargement of the prostate and a decrease in the size of the epididymis and a decrease in daily sperm production in male offspring. These effects of administration of bisphenol A to pregnant mice at doses of 2 and 20 μ g/kg/day were similar to effects of administration of DES to pregnant mice at 0.02 and 0.2 μ g/kg/day. These findings demonstrate that in the fetal prostate, bisphenol A is approximately 100-fold less potent than DES. In addition, bisphenol A and DES cause the same percent increase in prostate size relative to controls, revealing a similar efficacy of bisphenol A and DES in stimulating prostate enlargement.

It is important to note that the dose-response curve for estradiol and DES forms an inverted U. After the maximum increase in prostate size occurs (the maximum increase in prostate size is about 40% relative to controls), a further increase in dose results in a decrease in prostate size.

Frank Welsch and his colleagues at the Chemical Industry Institute of Toxicology (CIIT) exposed

pregnant and lactating Sprague-Dawley rats to bisphenol A in their drinking water (0, 0.005, 0.05, 0.5, 5.0 or 50 mg/L) from gestation day 2 to day 21 after birth of the litters. The authors estimated that the pregnant rats consumed BPA doses ranging from 0.001 to 10 mg/kg/day. In the published paper these authors concluded that there were no effects of BPA on preputial separation, anogenital distance, body weight or organ weight on the ventral prostate of male offspring. A panel selected by the US-NIH to review the low dose issue stated that reasons for this conclusion that there was no effect of bisphenol A were "flawed", "illogical" and "misleading". The NIH Low Dose Review Panel stated that there were significant effects of bisphenol A on ventral prostate weight at 10, 1000 and 10,000 μg/kg/day.

The above findings are important since the current LOAEL for bisphenol A accepted by the US-EPA is 50 mg/kg/day, and the daily dose considered safe for human consumption by the US-EPA is 50 μg/kg/day.

To determine whether effects of bisphenol A and other estrogens, such as DES, were directly on the prostate, Gupta placed the fetal mouse prostate in primary culture, and a dose of 50 pg/ml (50 ppt) stimulated prostate growth and gland formation, as well as androgen receptors, while a dose of 5 ppt resulted in no stimulatory effect. The effect of 50 ppt bisphenol A was similar to the effect of 0.5 ppt DES. Taken together, these findings provide additional evidence that in the fetal prostate, bisphenol A is approximately 100-fold weaker than DES.

These findings are important since it has been reported that the levels of unconjugated bisphenol A in human fetal serum is in the range of 0.1 - 10 ng/ml (0.2 - 9 ppb), and the mean bisphenol A concentration in human male fetuses is 3.5 ppb. The effects being reported in fetal mice and rats are thus occurring at doses below those found in human fetuses.

We exposed mouse fetuses to bisphenol A via administration of 20 μg/kg/day to pregnant mice. This resulted in a decrease in adult daily sperm production in the male offspring.

Adult Sprague-Dawley rats were administered a wide range of doses of bisphenol A. At doses of 20 μg/kg/day and above, bisphenol A reduced daily sperm production. However, the magnitude of the decrease in daily sperm production at 20 μg/kg/day was not different from the inhibition seen at 200 mg/kg/day (a 10,000-fold higher dose).

This finding is similar to the dose-response curve for effects of DES on daily sperm production in CF-1 mice exposed to DES only during fetal life, where daily sperm production was maximally reduced at a maternal dose of 0.01 μg/kg/day, which was the same as a dose of 1 μg/kg/day administered to pregnant females. The maximum amount that bisphenol A and other estrogenic chemicals can inhibit daily sperm production is by about 40% relative to controls. An important issue regarding the study by Sakaue is that bisphenol A shows the same effect on the adult testis as that observed in adults exposed to bisphenol A only during fetal life.

Effects of bisphenol A on the behavior of offspring at very low doses (2 and 20 μg/kg/day) administered to pregnant female mice have also been reported. Specifically, the duration of time that male offspring spent interacting with other males in an aggressive manner was significantly increased by both doses of bisphenol A. Other effects of exposure during development to low doses of bisphenol A on behavior in rats have also been reported.

Effects at very low doses of bisphenol A at 1 part per billion have been reported in studies of freshwater and marine snails by Oehlmann and colleagues. At doses down to the lowest tested in an initial set of studies (1 ppb), bisphenol A caused abnormalities in the reproductive organs and abnormal oocyte production in freshwater and marine snails. The marine snail Nucella lapillus was exposed in adulthood only, and males showed a decrease in penis and prostate gland length as well as stored sperm, again at the lowest dose tested (1 ppb). At a dose of 1 ppb bisphenol A also resulted in an increase in mortality in freshwater snails.

Effects of developmental exposure to bisphenol A on subsequent rate of growth and the timing of puberty have been reported. In CF-1 mice, pregnant females were administered a dose of 2 μg/kg/day bisphenol A. Bisphenol A resulted in female offspring entered puberty earlier than controls. A similar finding was reported by Iguchi and colleagues in CD 1 mice at a dose of 20 μg/kg/day. However, in the Howdeshell study offspring were placed with foster mothers at birth, and at weaning the male and female offspring exposed to bisphenol A during fetal life were heavier than controls. In contrast, in the Homma study, offspring remained with the treated mothers, and at weaning, bisphenol A-treated offspring were lighter than controls. The prediction that treatment of pregnant female mice with bisphenol A might result in a decrease in nursing behavior was confirmed in a study by Palanza. However, the decrease in nursing behavior caused by bisphenol A in the Palanza study did not result in a significant decrease in body weight of the pups. The basis for the differences in body weight in these studies thus remains to be determine, although an effect of bisphenol A on nursing likely plays some role.

There are a number of studies that have shown that the uterus in female rats and mice is markedly less sensitive to bisphenol A relative to the prostate in males. For example, we administered DES and bisphenol A to newborn CD-1 female mice via Silastic capsules for 5 days. At a dose of 0.01 μg/kg/day, DES significantly stimulated uterine growth. However, at doses up to 10,000 μg/kg/day (the highest dose administered), there was no effect of bisphenol A on uterine weight. In prepubertal CD-1 mice, a dose of 100 mg/kg/day was required to have a modest increase in uterine weight. These findings show that there are marked differences in the sensitivity of different tissues to bisphenol A. In contrast, the sensitivity of the prostate and uterus to DES was virtually identical.

Findings from a number of studies suggest that at a molecular level, the interaction of bisphenol A and estradiol with estrogen receptors is different. This provides the basis for a difference in the interaction of the estrogen receptor with proteins called coactivators. After binding to a chemical, the estrogen receptor regulates the rate of gene transcription through its association with coregulators. It is the overall balance of the relative expression levels of coactivators and corepressors that appears to be the important determinant of the tissue specificity of chemicals such as bisphenol A. Thus, the dose required for bisphenol A to elicit effects in one type of tissue cannot be used to predict the dose of the chemical that will elicit responses in other types of tissues.

There is now also extensive evidence that some effects of estradiol occur through the activation of cell signaling systems associated with receptors that are not located in the cell nucleus, and, instead, may be associated with the cell membrane. These effects are very rapid and occur in addition to the well studied effects mediated by receptors located in the cell nucleus, which take longer to occur. A characteristic of cell signaling systems is a very high level of amplification, with the result that a very low concentration of a compound can activate large changes in cell function. Recent studies have shown that bisphenol A can act via non-genomic (non-nuclear) receptors to activate cell signaling pathways at very low concentrations (1 nM or 228 parts per trillion, ppt), similar to estradiol.

The plastic manufacturers claim that the studies they have sponsored show no effects of bisphenol A. We have examined a the basis for differences in the outcome of fetal exposure to bisphenol A in previously conducted studies. These studies involved administration of bisphenol A to pregnant CF-1 mice and examination of male offspring during postnatal life. Of interest is that in the plastic industry study the prostate in control animals was significantly enlarged relative to control prostate weights reported in the study conducted in the vom Saal lab, which involved the use of the same strain of mouse at the same ages. One potential basis for the differences in body weight and prostate weight in the control males in these studies was that in the plastic industry study, a different animal feed was used relative to the feed used in the studies in the vom Saal lab. We thus compared the effects in CD-1 mice of exposure to the feed used by the plastic industry (Purina 5002) and the feed used in the vom Saal studies (Purina 5008 during

pregnancy and lactation and Purina 5001 maintenance diet after weaning).

Our findings show that adult male CD-1 mice exposed to the Purina 5002 feed had significantly more abdominal fat relative to males exposed to the Purina 5008/5001 feeds.

In addition, the males on the 5002 feed had prostates that were significantly (by about 40%) heavier than the prostates in males on the 5008/5001 feed. The 5002 feed thus appears to have resulted in the males being exposed to elevated levels of estrogen, yet the 5002 feed has lower levels of phytoestrogens than the 5008/5001 feeds.

We examined maternal and fetal blood for estradiol levels, and mothers and both male and female fetuses exposed to the 5002 feed had significantly elevated estradiol levels relative to animals on the 5008 feed.

Current methods of risk assessment are based on the assumption that the shape of the dose-response curve is always be monotonic. However, in experiments with hormones, drugs and other chemicals that act via hormonal mechanisms it is very common for the dose-response curve to be non-monotonic and form an inverted U, which in endocrinology is called a "biphasic" dose-response curve. In contrast, there appears to be a lack of awareness of this phenomenon in toxicology, as many toxicological studies in which effects only occur in a restricted "low dose" range, while the effect is not seen a lower or higher doses, conclude that there was no relationship between dose and response. Clearly, not all dose-response relationships are non-monotonic, but the fact that non-monotonic dose-response relationships do commonly occur in endocrinology has not been incorporated into the process of assessing risk of exposure to environmental chemicals. Thus, in risk assessment "safe" exposure levels are still calculated based on testing a few very high doses of a chemical and extrapolating from LOAEL or NOAEL doses using a linear extrapolation model; this involves dividing the LOAEL or NOAEL by 3-10 -fold safety factors to calculate a reference dose (RfD). If at the high doses tested, adverse effects that occur at low doses do not occur, this extrapolation procedure can result in calculation of a reference dose thousands of times higher than would be calculated based on testing a wide range of doses.

There are data from studies showing that adverse effects due to exposure to very low part per trillion and ppb doses of bisphenol A are not seen at much higher doses. For example, human prostate cancer cells were stimulated to proliferate in culture at a dose of 230 ppt, but a dose of 23 ppb produced no effect on cell proliferation, and there was an inverted-U dose-response curve.

Exposure of pregnant female CD-1 mice to 25 μ g/kg/day resulted in a significant increase in the length of mammary gland ducts at 30 days of age, while prenatal exposure to 250 μ g/kg/day resulted in a significant decrease in the length of ducts, and there was an inverted-U dose-response curve. Since in later adulthood both the 25 and 250 μ g/kg/day doses of bisphenol A resulted in larger mammary ducts, this suggests that there was a delay in development in females exposed to the 250 μ g/kg/day dose.

There was a significant increase in the number of embryos produced in freshwater snails at bisphenol A doses of 5 and 25 ppb, but not at 100 ppb, and again there was an inverted-U dose-response curve. These findings demonstrate that in a variety of model systems, bisphenol A, similar to other hormonally active chemicals, can produce inverted-U dose response curves.

The finding by Oehlmann and colleagues that the no effect concentration of bisphenol A in freshwater snails is 8 parts per trillion led to recommend that these findings should be considered in order to achieve environmental concentrations of bisphenol A that ensure the safety of wildlife, since concentrations of bisphenol A in river water are higher than the no effect level for snails.

In summary, there is extensive evidence for low-dose effects of bisphenol A in snails, fish, frogs, birds and mammals. Importantly, these effects occur with prenatal or postnatal exposure, including exposure just in adulthood. Adverse effects of bisphenol A occur at doses within the range of levels in women and human fetal blood.

Q&A

Morita: Thank you very much, Prof. vom Saal, very important work on low dose effect, especially the critical review of low dose effect and low dose effect mechanisms. Now we would like to accept comments or questions from the floor.

Becker: Thank you. Very nice presentation; I appreciate it this morning. I am Rick Becker from the American Chemistry Council.

Dr. vom Saal, to start I think it is unfortunate perhaps you were not at the IUPAC meeting last week or John Ashby could not be here at the meeting this week, because I think that neither myself nor anyone else could speak for John and his work, although just to point out that all of that work has been published in peer review literature, as has yours.

So it is really important when we have differences in a scientific community to look at that information on a weight of evidence basis. I think there is some agreement there about looking on a weight of evidence basis, as a comment.

One question I guess: you mentioned that one needed to look holistically at risk assessment from the standpoint of exposure, the background exposures, the existing levels of estrogen circulating as well as environmental estrogens.

So my question is have you considered human exposures to phytoestrogen, and where would bisphenol A or some of these other purported environmental estrogens fit in given the fact that some human diets are particularly rich in phytoestrogens? I am sure you are aware of the work of Dr. Safe and others to show that there are orders of magnitude, maybe 10,000 times higher exposure to phytoestrogens than to these chemicals.

And then, one last question: you made a comment, and I do not think it is supported by research. I just have not seen it yet if it is about enlarged prostate in the mice leading to the development of cancer. I do not recall seeing that published.

vom Saal: I did not say that. I want to clarify this so that it is very clear, because we are talking about biomarkers of risk. Elevated androgen receptors and elevated hormone responsiveness, whether you have elevated receptors or you have elevated hormone in the blood, you have a higher level of response, a higher level of proliferation, and that is clearly placing that individual, and this is very clear, for breast cancer and prostate cancer that would put you into a higher risk category.

It is a biomarker that is related to risk. It is not a causal pathway that we can identify. We do not know how this proliferation event occurring at a greater rate leads to a greater probability of a tumor, but that it happens is certainly documented.

I want to make it clear because the mouse that we are using is not a model for prostate cancer. But human prostate cancers, if you had a high level of androgen receptor, you would be more concerned about that individual. But you have raised many, many different issues.

These are the kind of data that Cagen and exactly John Ashby's data. I think if you raised the issue of weight of evidence that has nothing to do with what I am talking about. What I am talking about is understanding the mechanisms of how the data set was generated. What I am telling you is I can now generate an identical data set. I am now explaining the difference in their publication. I am not saying their data were not correct; in fact, I am saying I can replicate it. I want to emphasize that.

The reason I can replicate this finding of an abnormally enlarged prostate is that they fed the animals a food, 5002 and in the case of Ashby's lab, I do not have access to that food, but the animals had obviously more fat and they

had enlarged prostates. They look the same.

Any biologist who looks at the positive control and negative control being identical assumes they have contamination. That is why you run that group. Based on that hypothesis, we went to find where the source of the contamination was, and this relates to one of the questions you asked that the high phytoestrogen diet is very interestingly associated with the lowest endogenous estradiol.

I am sure you must be aware that there is a reasonably good literature out there showing that the phytoestrogens are actually inhibitory to estrogen synthesis and estrogen transport in the blood and this is exactly the prediction.

What is important from a life stage exposure point of view that I am afraid was beyond the issues raised by Steve Safe and I have had many conversations about this, is that if you take a postnatal female and remove her ovaries or prepubertally she has no estrogen, the phytoestrogens in a diet will have an estrogenic effect.

But during pregnancy when estrogen levels are very high, you get an inhibitory effect, and part of dealing with the endocrine disrupter issue, and hopefully this will be part of the discussion this afternoon, is babies are not little adults, pregnancy is a unique and very different physiological state, and the pharmacodynamics of bisphenol A in pregnancy and nonpregnancy are totally different.

The same is true for phytoestrogen, and the literature on phytoestrogens in adults or aging populations is irrelevant to what they do in a pregnant woman and her fetus. There is substantial documentation relating to that.

I am extremely interested in phytoestrogens because of these kinds of data and how they interact with exogenous chemicals like bisphenol A. We desperately need that kind of work. I am now doing that; in the future, I will be able to present data on that. I cannot write here; right now, there is a lot of speculation, and the one thing I am thinking is likely is that what will happen will not be exactly what we think. So I do not know if answered all your questions.

Becker: I think so. In terms of, and I think there is agreement there in terms of needing to look at the spectrum of estrogen types of exposures, whether it be endogenous, exogenous from foods phytoestrogen or exogenous from environmental contaminants. I think you need to get a whole picture in order to do the risk assessment. So I think there is agreement there.

vom Saal: I think that is a central part of my message here and I am very happy to see that we are totally on the same page on this. It also means that there can be tremendous confusion and misinterpretation of results if all of those factors are not taken into account.

This is part of creating a standard paradigm. We have to be very concerned with the model system, the types of food being used, the timing of exposure, the roots of exposure; all of these things become very critical because they can dramatically change the outcome.

I am not saying that anybody ever produced data that was not accurate. We are now beginning to understand at the mechanistic level how you can create differences when you do these experiments in slightly different ways, and from a biological perspective that is always very exciting.

Morita: Thank you very much. Time is up, I am sorry, so please ask questions after this session. Thank you very much, Prof. vom Saal.