



Memorandum



Date January 22, 2008
From Michelle L. Twaroski, Ph.D., Toxicology Group I, Division of Food Contact Notifications (HFS-275)
Subject Bisphenol A – Review of studies conducted by Vom Saal et al., Nagel et al., and MPI Research [circa 1997-1998]
Through Francis Lin, Ph.D.
Director, Division of Food Contact Notifications (HFS-275)
To Food Additive Master File (FMF) 580

During the recent review of data available to the agency on Bisphenol A (BPA, CAS RN. 80-07-7), a review by Dr. Bob Sprando authored in 1999, with the subject of “Review of MPI report on bisphenol A” and marked “DRAFT,” was identified in office files. This review focused on estrogenic compounds (BPA and controls) and their effects on male reproductive organs and was a review of several, at that time recent, reports by Vom Saal et al., Nagel et al., and a study performed by MPI Research that attempted to replicate the findings of the aforementioned authors (for references, see attached text). The MPI Research study is contained in FMF 580 and was completed in October 1998. The reviewed published studies are cited and summarized in the document, as well as other scientific data.

Dr. Sprando’s review summarizes the differences in the study protocols, findings and conclusions regarding the published studies and the MPI Research conducted study and concludes that the findings between the Vom Saal group [ca 1997-98] and petition contractor are in conflict. We believe the attached review of these data to be useful and appropriate for documenting the historical review record based on the data cited at the time the review was conducted. Considering the employee has since left the agency, we are accepting this “Draft” document as “Final”. The final date of the memorandum is the draft date of 02/04/1999.


Michelle L. Twaroski, Ph.D.


Attachment: Sprando/Biddle, Review of MPI report on bisphenol A, 02/04/1999



Memorandum

1999

Francis

Date February 4, 1999
From Bob Sprando
Subject Review of MPI report on bisphenol A.
To Kirk Biddle

Kirk,

Here is a draft of the review of the MPI document on bisphenol A. Please let me know what you think.

With regards,

Bob

DRAFT

BACKGROUND

The testis plays an essential role in the process of human reproduction. Nevertheless, little attention has been paid to the warnings coming from different sources pointing to the increasing incidence of male genitourinary abnormalities. Recent reports have suggested that semen quality has markedly decreased during the past 50 years and concomitantly the incidence of some genitourinary abnormalities, including testicular cancer, cryptorchidism and hypospadias has increased (1-6). Such remarkable change over a short period is more likely to be due to environmental contaminants rather than genetic factors. It is presumed that the increasing incidence of reproductive abnormalities in the human male may be related to increased estrogen exposure in utero, i.e. increased exposure to endogenous estrogens, synthetic estrogens and phytoestrogens. Epidemiological studies have shown that testicular cancer and cryptorchidism, the primary risk factor for human testicular cancer, are associated with a hormone imbalance during gestation and in particular, with increased maternal estrogen production in early pregnancy (7-11). The risk of testicular cancer is increased significantly in sons exposed to exogenous estrogens or high levels of endogenous estrogen in utero (9). Diethylstilbestrol (DES), the most extensively studied exogenous estrogenic hormone in human pregnancy, has been linked to a variety of structural and junctional alterations in utero exposed male genital tracts. The lesions ranged from minor structural changes such as epididymal or spermatocele cysts to major abnormalities

including hypoplastic testes, cryptorchidism, hypospadias and microcephalus with potential dysfunction such as ambiguous genitalia, male pseudohermaphroditism, infertility and testicular cancer (12-14). In human beings, DES appears to interfere with the normal hormonal function regulating spermatogenesis (12). The number of abnormal sperm in male offspring of women exposed to DES during pregnancy was much greater than that of control women. Also, in DES-exposed offspring, the average value for density and total motile spermatozoa ejaculated were less than 50% of those control subjects (12).

Growing evidence from animal experiments have confirmed that estrogens administered during critical periods of development result in long-term effects on the male reproductive tract. Perinatal DES exposure has resulted in abnormalities in development of the male genital tract (15). Prominent Mullerian remnants were observed in 97% of male mice exposed to 100 $\mu\text{g}/\text{kg}/\text{day}$ DES on days 9-12 (16) or 9-16 of gestation (16-17). When pregnant mice were treated with 100 μg DES/kg body weight on days 9 - 16 of pregnancy, 91% of treated male offspring had at least one undescended testis (16 - 17). The incidence of hyperplasia and adenocarcinoma of rete testes (18-20), epididymal cyst and inflammation (21-22) and testicular cancers (21) in treated offspring were also much higher than those of non-treated animals. Prenatal or neonatal exposure to estrogens other than DES, i.e. estradiol-17B (23,24), estradiol benzoate (25-28), ethinyl estradiol (29-31) or estradiol valerate (32), causes alteration in the male genital tract. Those

alterations include undescended testes, testicular hypotrophy, testicular teratoma, hyperplasia of Leydig cells, a metaplastic epithelium of periurethral region of the coagulating gland and hypotrophic prostate, seminal vesicles and other sex accessory glands (22-23). The developmental effects of estrogens on male sex accessory glands are time- and organ-dependent. When estradiol benzoate was administered neonatally, the organ weights and DNA content of seminal vesicles, ventral and dorsal prostates, epididymides and coagulating glands of treated animals decreased significantly as compared to controls. Similar but fewer extensive effects were observed in animals exposed to estrogens during puberty. No obvious changes were observed in prepubertally-treated animals (27). Neonatal estrogenization affected ventral prostate and seminal vesicles, by reducing their secretory function and the secretory protein level (27). A morphometric study showed that neonatal estrogen exposure decreased the volume of the glandular epithelium and increased the volume of the fibromuscular stroma in both ventral prostate and seminal vesicles (28). When rats were treated with estrogen neonatally the weight of the ventral prostate was lower in treated than in control rats at both 22 and 90 days of age. The weights of treated seminal vesicles and epididymides however, were increased on Day 22 of age and decreased on day 90 (18, 33). Neonatal treatment with estrogen and clomiphene also affected the development of blood-testis barrier in rats (25). Testosterone co-treatment partially counteracted some toxic effects of DES on the adult testis, but did not change sperm parameters and

fertility. There are two possible mechanisms by which estrogens affect the developing reproductive tract. Estrogens may disrupt male hormone secretions and metabolism. This alteration may exert its effect by acting directly on the hypothalamic-pituitary-testis axis to suppress the secretion of gonadotrophin and testosterone or by affecting the metabolism of androgens in the male reproductive tract (27). Additionally, estrogenization of the male genital tract may occur as a result of compounds in some way affecting either estrogen receptors (ER), androgen receptors (AR) or gonadotrophin receptors (35). Recently it has been suggested that exposure to low doses of estrogenic chemicals influences not only the development of male sociosexual behavior but also the size and functioning of the reproductive organs.

A considerable amount of attention has recently been focused on a wide variety of endocrine disrupting chemicals. One compound bisphenol A (BPA) has been reported to have estrogenic properties. BPA is an essential component of epoxy resins and is used in the lacquer lining of metal food cans (36), as a component of polycarbonates (37) and in dental sealants (38). As a result there is a considerable potential for human exposure. In particular there is the potential for exposure to pregnant women. It has been reported by Vom Saal et al., (39,40) and Nagel et al., (41) that maternal exposure to BPA at low doses during gestation can affect not only sperm count but the developing prostate in male offspring. As a result of these reports, MPI was contracted by The Society of the Plastics Industry, Inc. Bisphenol A Task Group to conduct a

study to evaluate the effects of bisphenol A (BPA) on male sexual development as measured by sex organ weights, daily sperm production, epididymal sperm count and testicular histopathology in the offspring of female mice exposed to the test article by deposition in the mouth on Days 11 -17 of gestation. The methodology utilized by MPI to investigate the effects of BPA on the male was that previously described by Vom Saal et al., (39,40) and Nagel et al., (41).

The following is a summary of the Vom Saal et al., (39,40) and Nagel et al., (41) papers as they relate to the MPI report.

Vom Saal F., Timms B.G., Montano M.M., Palanza P., Thayer K.A., Nagel S.C., Dhar M.D., Ganjam V.K., Parmigiani S. and Welshons W.V. 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc. Natl. Acad. Sci. 94:2056-2061.

Experimental Overview: Pregnant mice were exposed to DES (0.002, 0.02, 0.2, 2.0, 200-ng/g) in corn oil during days 11 - 17 of gestation or estradiol (0,100, 200, 300 μ g) dissolved in 20 μ l of sesame oil (day 13 - 19). The pregnant mice were exposed to DES orally (not gavage) while estradiol was administered to via silastic capsules. F1 generation males from mothers treated with DES were weaned on day 23 and euthanized at approximately 8 months. Pregnant females exposed to estradiol were killed on day 19 and 1MF

males (male fetuses in the uterus located between 1 male and 1 female fetus) were identified and reared with litter mates by foster mothers. These F1 generation males whose mothers were exposed to estradiol were weaned on day 23 and euthanized at approximately 8 months.

Results: Low dose estrogen treatment: 1) increased the number of prostatic buds; 2) caused the number of prostatic buds to increase along a greater length of the urogenital sinus relative to control males and 3) increased prostate size and the number of androgen receptors.

Low dose DES treatment significantly increased adult prostate weight in 0.02, 0.2, 2.0-ng/g treatment groups compared to controls. No effects were observed at 0.002 ng/mg DES. High dose DES treatment (200 ng/g) significantly reduced prostate weight compared to controls.

Vom Saal F.S., Cook P.S., Buchanan D., Palanza P., Thayer K.A., Nagel S.C., Parmigiani S., and Welshons W.V. 1998. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of the reproductive organs, daily sperm production and behavior. Toxicology and Industrial Health 141(1/2):239-260.

Experimental Overview: Bisphenol A or octylphenol at a concentration of 2 ng/g or 20 ng/g were dissolved in corn oil and

fed to pregnant mice during gestation days 11 - 17. F1 generation males were weaned at 23 days and euthanized at 6 months. At 6 months the animals were weighed and the testes, epididymides, preputial glands and seminal vesicles were removed and weighed. Daily sperm production and the efficiency of sperm production were calculated. The prostate was removed and weighed. Information regarding the prostate can be found in Nagel et al. (41).

Results: A 2 ng/g dose of bisphenol A: 1) permanently increased the size of the preputial glands and 2) reduced the size of the epididymis. A 20 ng/g dose of bisphenol A significantly decreased the efficiency of sperm production.

A 2 ng/g dose of octylphenol reduced daily sperm production and the efficiency of sperm production.

Nagel S., Vom Saal F., Thayer K., Dhar M.G., Boechler M. and Welshons, W.V. 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ. Health Perspect. 105(1):70-76.

Experimental Overview: The Nagel and colleagues have developed a relative binding affinity-serum modified access (RBA-SMA) assay which can be used to determine the effect of serum on the access of xenoestrogens to estrogen receptors within intact cultured MCF-7 human breast cancer cells. In the present study, the assay

indicated that, relative to estradiol, serum had less of an inhibitory effect on the cell uptake and binding in MCF-7 cell of bisphenol A, while serum had an inhibitory effect on octylphenol relative to estradiol. Extrapolation of the relative activities in adult serum predicted that the relative estrogenic activity of bisphenol A would be 500 times greater than that for octylphenol in the fetal mouse. As a result of these findings, bisphenol A or octylphenol at a concentration of either 2 ng/g or 20 ng/g were dissolved in corn oil and fed to pregnant mice during gestation days 11 - 17. F1 generation males were weaned at 23 days and euthanized at 6 months. At 6 months the animals were weighed. The prostate was removed and weighed.

Results: Exposure of male mouse fetuses to either dose of bisphenol A but to neither dose of octylphenol significantly increased their adult prostate weight relative to control males. This finding was consistent with the higher predicted bioactivity of bisphenol A than octylphenol in the RBA-SMA assay.

COMPARISON OF THE EXPERIMENTAL DESIGNS

Table 1 presents a comparison of the experimental design utilized by MPI, Vom Saal (39,40) and Nagel et al., (41). Several major differences were observed when the experimental designs and results were compared. These include:

1. The strain of animal utilized in the study:

CF-1 mice obtained from Charles River were utilized in each study, however the CF-1 mice utilized by Vom Saal et al., (39, 40) and Nagel et al., (41) were obtained from a breeding colony at the University of Missouri-Columbia. The animals used to start this colony were originally bought from Charles River in 1979. The breeding colony has since been maintained as an outbred stock in a closed colony.

2. The number of animals utilized in the studies.

The MPI study utilized a larger number of animals (n = 18 - 26 mice/group) than the Vom Saal et al., (39, 40) and Nagel et al., (41) studies (n = 5 - 8 mice/group, respectively).

3. The age of the F1 males animals at euthanasia.

The F1 generation male mice in the MPI study were euthanized on day 90 while the F1 generation male mice in the Vom Saal et al., (39, 40) and Nagel et al., (41) studies were euthanized at approximately 180 days (BPA + Controls) or 240 days (DES + Controls)

4. The inability of the DES positive control to induce a positive effect.

An effect on prostate weight, previously reported by Vom Saal (see Table 1), was not obtained when DES was utilized as a positive control in the MPI study.

5. Conflicting results when the studies (MPI v.s. Nagel et al., and Vom Saal et al.,) were compared.

The results from the MPI study suggested: 1) BPA had no effect on prostate weight, other reproductive organ weight, sperm count or daily sperm count and 2) DES had no effect on prostate weight. In striking contrast the Vom Saal et al. (39, 40), study results suggested that exposure to BPA at: 1) 2ng/g increased size of preputial glands and reduced size of seminal vesicles and epididymides; 2) 20ng/g reduced efficiency of sperm production; 3) 2ng/g and 20ng/g increased adult prostate weight. Additionally, maternal exposure to DES at a concentration of 0.2 μ g/kg (200ng/g) resulted a reduction in the size of the prostates in F1 males.

DISCUSSION

The MPI study was designed to replicate the work of Vom Saal and Nagel and either confirm or refute their findings. A careful comparison of the experimental designs shows several areas where the MPI study differed from the Vom Saal and Nagel studies. These included the: 1) animal model utilized; 2) number of animals utilized per experimental group 3) failure of the positive control to induce an effect and 4) age of the animals at the time of sacrifice.

Animal Model Utilized: Since the CF-1 mice utilized by Vom Saal were obtained from a colony of CF-1 mice which have been inbred for approximately 20 years it is possible that the Charles River CF-1 mice used by MPI were not genetically the same as the Vom Saal animals. It is possible that the Vom Saal animals are more sensitive to estrogenic mimics than the Charles River animals. It would have been interesting if MPI could have obtained some animals from Vom Saal and run these animals concurrently with their animals to determine if the animals responded similarly.

Animal Numbers: The MPI study utilized large numbers of animals per group compared to that utilized by Vom Saal and Nagel. As a consequence, the MPI study is statistically more powerful. It is difficult to speculate what would occur if Vom Saal repeated his studies with larger numbers of animals per experimental group.

Positive control: Although the MPI study utilized a dose of DES which was previously shown by Vom Saal to induce a positive effect on the prostate, it concerns me that the positive control did not produce any effect in the MPI study. It would have been advantageous if MPI would have used either an additional higher dose or a single higher dose to insure that a positive effect was obtained.

Age of F1 generation at males at euthanasia: The F1 generation males utilized in the Vom Saal studies were 2 - 3 times older than the animals used in the MPI study. It is possible, but unlikely, that the results obtained by Vom Saal would have been obtained in the MPI study if the MPI animals would have been euthanized at a later date. Again the genetic make-up of the Vom Saal animals is unknown.

CONCLUSIONS

It is obvious that Vom Saal and the petition contractor have come to different conclusions concerning the compound. There are differences in how these tests were performed. One procured the animals directly from Charles River, and the other has a colony of animals derived from Charles River. There is a difference of power between the two studies. If the study is well conducted, we normally have to accept the most sensitive study. The only way around this that I can see would be to run a third study in which the petitioner would obtain animals from Vom Saal and use his animals also. It would be best if Vom Saal would do the same, such that there could be some consensus as to the SOPs used and the power of the study. Hopefully, a single answer would surface.

Table 1. Comparison of the experimental design of the MPI study and the Vom Saal et al., 1998 study.

	MPI	Nagel et al., 1997 Vom Saal et al., 1997 Vom Saal et al., 1998
Strain	CF-1 Strain: Charles River; Portage, MI	CF-1 Strain; Charles River; Purchased 1979 and maintained as outbred stock in a closed colony.
Test Compound	BPA: 0.2, 2.0, 20.0 and 200 µg/kg	BPA: 2.0 and 20.0 µg/kg (ng/g)
Positive Control	DES: 0.2 µg/kg	DES: 0.002-, 0.02-, 0.2-, 2.0-, 20-, 200 µg/kg (ng/g) (Vom Saal et al., 1997)
Negative Control #1	Tocopherol-stripped corn oil.	Tocopherol-stripped corn oil.
Negative Control #2	Unhandled Control (?)	Unhandled Control
Dams/group		
BPA:	28/group	7 females/group
DES:	28/group	6-8 females/group (Vom Saal et al., 1997)
Negative Control #1	28/group	6 females/group (received corn-oil)
Negative Control #2	28/group (unhandled control ???)	5 females/group (unhandled control)
Culling	Yes Litters reduced to 8 pups (8 males when possible) per litter.	?

	MPI	Nagel et al., 1997 Vom Saal et al., 1997 Vom Saal et al., 1998
Weaning	Day 22 of lactation	Day 23 of lactation Male litter mates housed 3/cage until 5 months. At 5 months selected males housed individually. (n=?/group)
F1 males:		
BPA	0.2 BPA (23 pups); 2.0 BPA (22 pups); 20 BPA (18 pups); 200 BPA (24 pups)	5-7(?) males/group
DES	0.2 DES (23 pups)	6 - 8 males/group (Vom Saal et al., 1997)
Negative Control #1	18 pups	
Negative Control #2	26 pups	8 males
Diet		
BPA+Controls	#5002 Certified Rodent Chow; Water - ad libitum	#5001 Certified Rodent Chow-Weanlings; #5008 Certified Rodent Chow- For Pregnant and Lactating Females; Water - ad libitum
DES+Controls	#5002 Certified Rodent Chow; Water - ad libitum	Standard Purina Lab Chow (Vom Saal et al., 1997) Water - ad libitum
Temperature Humidity	21 - 24 °C 43 - 65%	21 - 24 °C ?
Light/Dark	12/12	12/12
Method of Delivery	Deposition in mouth by micropipetter.	Deposition in mouth by micropipetter.

	MPI	Nagel et al., 1997 Vom Saal et al., 1997 Vom Saal et al., 1998
Treatment Period	gestation days 11 - 17	gestation days 11 - 17
Age at euthanasia	90 days (3 months)	BPA + ctrls: 6 mo. DES + ctrls: 8 mo. (Vom Saal et al., 1997)
Parameters Evaluated		
BPA	Weights: Testis, epididymis, preputial glands, seminal vesicles, prostates. Sperm: DSP, cauda epididymal sperm numbers	Weights: Testis, epididymis, preputial glands, seminal vesicles. Sperm: DSP (rt. testis), efficiency of sperm production. n=5 males/experimental group; 8 controls.
DES	Weights: Testis, epididymis, preputial glands, seminal vesicles, prostates. Sperm: DSP, cauda epididymal sperm numbers	Prostate Weights (Vom Saal et al., 1997)

	MPI	Nagel et al., 1997 Vom Saal et al., 1997 Vom Saal et al., 1998
Results	<p>BPA: No effect on prostate or other reproductive organ weights, sperm count or daily sperm count.</p> <p>DES: No effect on prostate weight</p>	<p>BPA: 2ng/g: increased size of preputial glands and reduced size of epididymides.</p> <p>20ng/g: decreased efficiency of sperm production</p> <p>2ng/g and 20ng/g: increased adult prostate weight</p> <p>DES: small prostates (0.2µg/kg = 200ng/g)</p>

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