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Reproductive Toxicology

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Letter to the Editor

Reproductive effects of the parabens

The paper on possible parabens endocrine effects by Boberg et al. [1] provided an ambitious review of the literature on exposure and toxicology studies for these compounds; however, the literature does not support the conclusion of these authors that the safety margin for propylparaben is “very low” based on reproductive studies in rats and mice.

1. Estrogenicity

Part of the concern expressed by Boberg et al. is predicated on the putative estrogenicity of the parabens and their common metabolite, *p*-hydroxybenzoic acid. Estrogenicity of the parabens has been suggested by *in vitro* estrogen receptor binding studies, as summarized in Table 4 of the Boberg et al. paper. Methyl-, ethyl-, *n*-propyl-, and *n*-butylparabens were found to be about 10^{-4} times as potent as 17β -estradiol in binding the estrogen receptor in a yeast-based assay [2]. In an MCF-7 breast cancer cell proliferation assay, these parabens were 10^{-5} to 10^{-6} times as potent as 17β -estradiol, and the common metabolite, *p*-hydroxybenzoic acid, was inactive [3].

The most commonly used *in vivo* test of estrogenicity has been the uterotrophic assay. Boberg et al. summarized the parabens uterotrophic assays in their Table 3. Note that all positive assays for the alkylparabens were performed using subcutaneous administration. Oral administration of these agents has not demonstrated estrogenic activity in the uterotrophic assay [4]. The importance of route of administration is likely due to the hydrolysis of parabens in the skin and liver resulting in limited systemic concentrations of these agents after oral or transdermal administration (reviewed in [5]). Subcutaneous administration bypasses the portal of entry hydrolysis in skin and gastrointestinal tract, allowing greater internal exposure to parent compound.

Even accepting the apparent estrogenicity of subcutaneous *n*-butylparaben in some screening tests, this finding is not necessarily of toxicological significance as implied by Boberg et al. Shaw and deCantanzaro reported that up to 35 mg/CF-1 mouse (about 1000 mg/kg bw) daily for the first four days of gestation did not prevent implantation, a sensitive measure of estrogen toxicity [6]. Boberg et al. cite this study, but focus on the apparent positive uterotrophic response of this dose in ovariectomized CF-1 (but not CD-1) mice. The lack of prevention of implantation is arguably more toxicologically relevant than the 22% increase in wet weight of the uterus reported in the CF-1 mice.

The estrogenicity analysis of Boberg et al. used reported human blood concentrations of *n*-butylparaben, *n*-propylparaben, and *p*-hydroxybenzoic acid and estimates of estrogenic potency of each compound to arrive at a 17β -estradiol-equivalent concentration. The *n*-butylparaben concentration was taken from the

study of Janjua et al. [7] in which healthy men were treated with close to total body topical application of a cream containing 2% each diethyl phthalate, dibutyl phthalate, and *n*-butylparaben. The cream was applied at 2 mg/cm² body surface area for a total dose of 34–48 g/subject each day. It appears unlikely that the study of Janjua et al. adequately models the exposure of children to *n*-butylparaben in topical products. The study cream was applied over the entire body except for scalp and genitalia and left undisturbed for 20 min before the subjects dressed. The *n*-butylparaben content of the cream was 2%, or 5 times the permissible limit of 0.4% in consumer products. Moreover, the *n*-butylparaben was administered in a cream with high concentrations of phthalates that compete for the same hydroxylases as the parabens.

The analysis for *n*-propylparaben assumed a blood concentration of 2.3 ng/mL. This concentration was cited as based on measurements in 15 banked serum samples analyzed by Ye et al. [8]; however, Ye et al. reported a mean *n*-propylparaben concentration of 0.4 ng/mL with a median concentration below the limits of detection. The 2.3 ng/mL value was the upper end of the range of detected concentrations. The 15 sera were obtained from adult men and women, and it is not known whether the extreme value from this study is achievable in children.

The statement by Boberg et al. that *p*-hydroxybenzoic acid is estrogenic is based on a paper by Lemini et al. [9]. This study found a uterotrophic effect of subcutaneous *p*-hydroxybenzoic acid in CD-1 mice but not in Wistar rats. The estrogenic potency compared to 17β -estradiol was five-fold greater after a 50 mg/kg dose than after a 150 mg/kg dose, raising a question about the reliability of the study. Other investigators have reported *p*-hydroxybenzoic acid to be inactive in a yeast based estrogen screen [2] and in the uterotrophic assay in mice [4,5] and rats [4]. The concern expressed by Boberg et al. about the estrogenicity of this common metabolite does not appear justified.

2. Toxicology studies

The use of toxicology tests with apical end points can be expected to be more useful in risk assessment than are estrogenicity screens. Boberg et al. rely on male reproductive studies by Oishi for the NOEL for *n*-propyl- and *n*-butylparaben of 10 mg/kg bw/d (given in the diet) [10,11]. They dismiss the study of Hoberman et al. [12], which tried unsuccessfully to reproduce the Oishi results for *n*-butylparaben. In dismissing the Hoberman study, Boberg et al. indicate that the European Scientific Committee on Consumer Products (SCCP) concluded that shortcomings of the study prevented it from being considered valid. The shortcomings identified in the SCCP opinion [13] and my comments are listed here:

Criticism	Comment
The study did not follow a scientific protocol—no OECD number, no Annex V EC B. number.	The study was designed to replicate the Oishi protocol.
There was no information on which pups were from the same dam.	The published paper identifies two males from each litter as having been selected for each dose group.
The body weights of the animals were “very divergent.”	The distribution of body weights was not given in the published paper.
Reported hormone levels were characterized by large standard deviations.	Testosterone, FSH, and LH were measured in four dose groups at each of weeks 3, 5, 7, and 9 for a total of 48 measurements. Of these, two testosterone means have a coefficient of variation of around 80%. The remainder are in the 20–60% range, not unusual for hormone measurements. According to the U.S. Environmental Protection Agency, acceptable coefficients of variation for serum testosterone in rats of this age are up to 89.7% [14].
The sampling times were not given, so diurnal variation in hormone concentrations cannot be considered.	The published paper indicates that samples were taken at the same time of day each week between 8:30 and 11:00 AM.
Many animals displayed unexpected clinical symptoms, such as chromorhinorrhea and chromodacryorrhea, which raised questions about their general health at the start of the study.	The published paper indicates that animals were examined for general health status prior to the study.
Nearly all findings that have statistical significance were “waived” due to the lack of dose-dependency and abnormal high values in control animals.	It is standard to identifying these types of potential errors in toxicology studies.

The high control value for serum testosterone in week 3 was identified as being due to two animals in this group. Even if the week 3 control outliers are kept in the analysis, the *n*-butylparaben NOEL for this end point would be 10 mg/kg bw/d. The putative finding of a decrease in serum testosterone in *n*-butylparabens-treated animals at week 3 was not supported by testosterone findings at weeks 5, 7, or 9, but a conservative reading of this study could admit a NOEL as low as 100 mg/kg bw/d. There was also an increase in abnormal sperm at 100 and 1000 mg/kg/d, supporting the conservative NOEL of 10 mg/kg/d.

The Oishi studies on *n*-propyl and *n*-butylparabens, which identified a LOAEL of 10 mg/kg bw/d, reported control values for serum testosterone and for sperm concentration that were well above typical values for these end points in Wistar rats. The National Toxicology Program reported the 95th percentile for epididymal sperm concentration in control rats at $1068 \times 10^6/g$ based on 25 studies [15], whereas the Oishi control rats had a mean epididymal sperm concentration of $1698 \times 10^6/g$ in the *n*-butylparaben study and $1080 \times 10^6/g$ in the *n*-propylparaben study. Acceptable control serum testosterone concentrations in Wistar rats of the age used in this study are up to 2.540 ng/mL [14]. The control means in the Oishi *n*-propyl- and *n*-butylparabens studies were about 9 ng/mL. It appears, then, that the Hoberman et al. study constitutes a more reliable assessment than the Oishi studies, and the failure of Hoberman et al. to confirm Oishi is an important factor in deciding how much weight to put on the Oishi studies [16].

Other studies that bear on the reproductive and developmental effects of *n*-propyl- or *n*-butylparaben include a female pubertal assay in rats given parabens by gavage that showed no effect of *n*-propyl- or *n*-butylparaben on vaginal opening, uterine weight, or estrous cyclicity at up to 1000 mg/kg bw/d [17]. Histologic changes in the ovary and uterus were observed at an *n*-butylparaben dose

of 62.5 mg/kg bw/d and an *n*-propylparaben dose of 1000 mg/kg bw/d. The histologic changes are of questionable toxicological significance given the lack of effect on estrous cyclicity and uterine weight. A developmental study used subcutaneous *n*-butylparaben at 100 or 200 mg/kg bw/d in pregnant rats during gestation and lactation [18]. A dose of 100 mg/kg bw/d was associated with a decrease in live pups at birth and 200 mg/kg bw/d was associated with a reduction of survival to weaning. There were no alterations in anogenital distance on postnatal day 1. Vaginal opening was advanced at 100 but not at 200 mg/kg bw/d. Changes in weights of male reproductive organs were reported but were not dose-related except possibly for an increase in testis weight at 200 mg/kg bw/d on postnatal day 90. Epididymal sperm concentration and motility were decreased by both doses of *n*-butylparaben. A standard developmental toxicity study of *n*-butylparaben by gavage in rats showed no developmental effects at dose levels up to 1000 mg/kg bw/d in spite of maternal toxicity at 100 mg/kg bw/d [19].

Considering the toxicology data base as a whole, a NOEL of 10 mg/kg bw/d and a LOAEL of 100 mg/kg bw/d for oral exposure to *n*-butylparaben appear conservative. This compound is usually considered to be the most potent of the commonly encountered *n*-alkylparabens (based largely on in vitro studies of estrogenicity). Boberg et al. have adopted the human exposure estimates of Cowan-Ellsberry and Robison [20] of 0.34 mg/kg bw/d for *n*-propylparaben and 0.0016 mg/kg bw/d for *n*-butylparaben. Using these estimates, the margins of exposure are 29 for *n*-propylparaben and 6250 for *n*-butylparaben, which are very reassuring, indeed, for compounds that are considerably hydrolyzed at their portals of entry for typical human exposures.

Disclosure

Dr. Scialli is a consultant for Proctor & Gamble.

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5 January 2011

1 March 2011

11 March 2011

Available online xxx