Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/etap

# Effects of *n*-butylparaben on steroidogenesis and spermatogenesis through changed $E_2$ levels in male rat offspring

*Linyuan Zhang*[a,1], *Li Dong*[b,1], *Sijin Ding*[a], *Peihuan Qiao*[a], *Chong Wang*[b], *Ming Zhang*[b], *Lixia Zhang*[b], *Qingcheng Du*[a], *Yimin Li*[b], *Ning Tang*[b], *Bing Chang*[a,*]

[a] *Key Laboratory of Chemical Safety and Health, National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, No. 29 Nanwei Road, Xicheng District, Beijing 100050, China*

[b] *Institute for Environmental Health and Related Product Safety, Chinese Center for Disease Control and Prevention, No. 7 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China*

ARTICLE INFO

*Article history:*
Received 2 September 2013
Received in revised form
25 January 2014
Accepted 26 January 2014
Available online 12 February 2014

*Keywords:*
Parabens
Endocrine disrupting chemicals
Reproduction
Testosterone
Oestrogene

ABSTRACT

Parabens are widely used as antibacterial agents, which are concerned recently in the relationship between the use of parabens and reproductive toxicity. So that reassessment of the risk of parabens is needed. In this study, one of parabens, *n*-butylparaben (*n*-BP) was orally administered to pregnant Wistar rats (0, 64, 160, 400 and 1000 mg/kg/day) from gestation day (GD) 7 through postnatal day (PND) 21. Reduced anogenital distance (AGD) and delayed preputial separation (PPS) were observed in the male offspring. The weights of the testes were significantly reduced at PND 21–90. The weights of the epididymides were significantly reduced at all monitoring points, except PND 35. Seminal vesicle weights were significantly reduced on PND 21. Serum testosterone (T) was significantly decreased, especially on PND 49. The levels of 17β-estradiol ($E_2$) showed an increase at each of the tested points except on PND 180. Serum luteinising hormone (LH) and follicle-stimulating hormone (FSH) levels in the *n*-BP treated groups were lower on PND 21, 35 and 49 but elevated on PND 90 compared to control levels. *n*-BP reduced epididymal cauda sperm counts and daily sperm production in a dose-dependent manner; this difference was statistically significant at exposure groups of 400 and 1000 mg/kg/day. The present study strongly suggests that exposure to *n*-BP *in utero* and during lactation has adverse effects on the reproductive system in male offspring, with

*Abbreviations:* *n*-BP, *n*-butylparaben; GD, gestation day; PND, postnatal day; EDCs, endocrine disrupting chemicals; AGD, anogenital distance; TD, testicular descent; PPS, preputial separation; DSP, daily sperm production; BPA, bisphenol A; DES, diethylstilbestrol; ATZ, atrazine.

* Corresponding author. Tel.: +86 10 83132559; fax: +86 10 83132559.
E-mail addresses: jessica242@sina.com (L. Zhang), lizi1214@sina.com (L. Dong), dsj1348@126.com (S. Ding), qiaopeihuan@aliyun.com (P. Qiao), wangchongfly@163.com (C. Wang), zhangming2550@163.com (M. Zhang), xia007123@sina.com (L. Zhang), 1329272221@qq.com (Q. Du), yiminli@sina.com (Y. Li), ningtanglcp@126.com (N. Tang), bjchangbing@sohu.com (B. Chang).

[1] These authors equally contributed to the work.



http://dx.doi.org/10.1016/j.etap.2014.01.016

a no observed adverse effect level (NOAEL) of 160 mg/kg/day. To our knowledge, this is the first study that reports increased $E_2$ levels of male rats following *n*-BP exposure; we suggest that $E_2$ levels may be considered as biomarkers for some endocrine disrupting chemicals (EDCs).



## 1. Introduction

Parabens are a group of alkyl ester compounds present in *p*-hydroxybenzoic acid that are widely used as antibacterial agents/preservatives in cosmetics, personal care products, pharmaceuticals and food. People may be exposed to parabens through inhalation, dermal contact, and ingestion (El Hussein et al., 2007; Soni et al., 2005). Formerly, parabens were thought to be relatively safe compounds with low toxicities and bioaccumulation. However, the literature has increasingly shown that parabens possess weak oestrogenic properties in *in vitro* and *in vivo* uterotrophic assays (Lemini et al., 2003; Routledge et al., 1998).

Data from some studies on male rodents subjected to parabens challenges have suggested that the function of the testes was impaired during the challenge, leading to low sperm counts and testosterone (T) levels (Kang et al., 2002; Oishi, 2001, 2002a,b). Epidemiological studies have suggested that parabens and related metabolites can be measured in human blood and urine (Janjua et al., 2007, 2008; Ye et al., 2006, 2008). Darbre et al. (2004) found that intact parabens could be detected in human breast tumour tissues. Although there was no conclusive evidence to establish the relationship between the use of underarm cosmetics containing parabens and breast cancer (SCCP, 2005), risk assessments and determination of the margins of safety concerning parabens are still needed; furthermore, an available and precise lowest observed adverse effect level (LOAEL) and no observed adverse effect level (NOAEL) of parabens is required from animal studies.

For male reproduction toxicity endpoints, the industry and the cosmetic ingredient review panel (CIR) agree on a NOAEL for all parabens at 1000 mg/kg/day, which is based on a study by Hoberman et al. (2008). The European Food Safety Authority (EFSA) use this value for methylparaben and ethylparaben (EFSA, 2004), but this value is not used for propylparaben due to the lack of relevant available data for this compound. However, a report by the Danish National Food Institute established 10 mg/kg/day as a LOAEL for propylparaben based on a study by Boberg et al. (2009,2010). Considering the scientific acceptability of parabens, the Scientific Committee on Consumer Products (SCCP) conservatively chose the no observed effect level (NOEL) value of 2 mg/kg/day for propylparaben and butylparaben (*n*-BP) based on the research by Fisher et al. (1999). Still, due to the number of insufficient and divergent repeated dose studies (Fisher et al., 1999; Hoberman et al., 2008; Oishi, 2001, 2002a), it is hard to determine a robust NOAEL value for the observed reproductive effects of butylparaben or propylparaben in rodents. This lack of agreement makes it difficult to select and implement appropriate regulatory responses to the hazards of parabens.

Previous researchers have mainly focused on changes at the level of serum testosterone in a model of male reproduction. In females, research has focused on changes in serum estradiol levels, as 17β-estradiol ($E_2$) and T correspondingly play vital roles in the development of sexual differentiation and phenotype. Indeed, in the 1930s, an oestrogen-related hormone was detected in the urine of stallions (Zondek, 1934), and 30 years later, $E_2$ biosynthesis was first discovered in the human testis (Jayle et al., 1962). More recently, the presence of large quantities of oestrogen in the rete testis fluid and spermatic veins of numerous mammals has been reported (Hess, 2003). The concentrations of oestrogen in the testis and rete testis fluids far exceed the concentration in the serum of males of various species (Hess, 2000). This suggests a central role for oestrogen in testicular function. An elementary physiological role for oestrogen in male fertility was not identified until the early 1990s, although the administration of xenoestrogens during foetal and neonatal development has been reported to be associated with a series of male reproductive disturbances, such as cryptorchidism, epididymal defects, impaired fertility, and an elevated incidence of testicular cancer (Gill et al., 1979; Jensen et al., 1995; McLachlan et al., 1975). Infertility in mice with a functional oestrogen receptor-α knockout (ERαKO) was the first demonstration that oestrogen was essential for male fertility (Eddy et al., 1996; Hess et al., 1997; Korach, 1994; Lubahn et al., 1993); this phenotype was possibly due to a defect in efferent ductule development or function (Hess et al., 1997; Lee et al., 2000). Mice lacking a functional aromatase gene (aromatase knockout, ArKO) were also infertile; however, this appears to be due to a specific defect in germ cell development (Robertson et al., 1999). Further explanation of the role of endogenous oestrogens when xenoestrogen exposes in male reproductive tract is needed.

As far as steroid concentrations are concerned, the evidence for the importance of a balance between androgens and oestrogens on human was well reviewed (Williams et al., 2001). It has been suggested that this balance may be important in maintaining normal spermatogenesis (Zhang et al., 2010). Therefore, we conducted measurements not only of hormone T but also of serum estradiol hormones among male offspring on PND 21, 35, 49, 90, and 180. We also performed necropsy examinations to observe the dynamic changes in the developing testes. The present study chose the *in utero* and lactational exposure, which recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), since the foetal and neonatal periods represent a critical window during which organisms are particularly sensitive to endocrine active substances (McLachlan et al., 2001; Sharpe et al., 1995; Sharpe and Skakkebaek, 1993; Welsh et al., 2008 ).