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Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model

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ABSTRACT

In this study, a female pubertal assay on the effects of parabens, including methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutylparaben, was performed in a female Sprague–Dawley rat model during the juvenile-peripubertal period. The rats were orally treated with these parabens from postnatal day 21–40 in a dose-dependent manner (62.5, 250 and 1000 mg/kg body weight [BW]/day). 17α-Ethinylestradiol (1 mg/kg BW/day) was used as a positive control and corn oil as a vehicle. A high dose of methyl- and isopropylparaben (1000 mg/kg BW/day) resulted in a significant delay in the date of vaginal opening and a decrease in length of the estrous cycle. In measurements of organ weight and body weight, we observed significant weight changes in ovaries, adrenal glands, thyroid glands, liver, and kidneys; conversely, body weight was not altered following paraben treatment. The potential effects of parabens on estrogenicity were shown in histopathological abnormities in the reproductive organs. Histological analysis of the ovaries from the peripubertal rats revealed a decrease of corpora lutea, increase in the number of cystic follicles, and thinning of the follicular epithelium. In addition, morphological studies of the uterus revealed the myometrial hypertrophy by a high dose of propyl- and isopropylparaben (1000 mg/kg-day), and in all dose groups of butyl- and isobutylparabens. However, no significant histopathological changes were observed in the other organs (i.e. adrenal and thyroid glands). We also observed a significant decrease in serum estradiol and thyroxine concentrations in methyl-, ethyl-, propyl-, isopropyl-, and isobutylparaben-treated groups. A receptor-binding assay indicated that the relative binding affinities of parabens to estrogen receptors occurred in the order: isobutylparaben > butylparaben > isopropylparaben = propylparaben > ethylparaben. These values were much lower than the binding affinity for 17β-estradiol. Taken together, long-term exposure to parabens, which show less estrogenic activity than estradiol, can produce suppressive effects on hormonal responsiveness and can disrupt the morphology of reproductive target tissues. In addition, the relation between thyroid weight and thyroid hormone may influence circulating levels of parabens, suggesting the effects of parabens as thyrotoxic during this critical stage of development in female rats.

1. Introduction

A wide range of parabens including methyl-, ethyl-, butyl-, isobutyl-, and isopropylparaben are widely used as antimicrobial agents in food ingredients, cosmetic consumer products such as underarm deodorants, antiperspirants, skin moisturizers, body creams, body sprays and suncare products, or in pharmaceutical preparations [1,2]. Recently, parabens have been shown to act as xenoestrogens, a class of endocrine disruptors (EDs), whose chemical structures can be closely associated with differences in their estrogenicity. Like other xenoestrogens, parabens can also mimic the effects of physiological estrogens. They may bind to estrogen receptors (ERs), stimulate the ER-dependent response, and/or influence the expression of estrogen-responsive genes, including ERα, the progesterone receptor (PR) and pS2 [3,4]. ER-dependent estrogenic activities of parabens have been demonstrated in the MCF-7 human breast cancer cell line [3–6] and ZR-75-1 cell lines [5,7] in immature and adult mice and rats [8–14] and in fish [15].

The potency of parabens as estrogens appears to depend on the lengths of their alkyl side chains [4,13]. An increase in the size of the alkyl group may enhance paraben transactivation of ERs *in vitro* [16]. Parabens can mimic the effects of the main natural estrogen (17β-estradiol) by binding to ERs [4]. In addition, parabens may increase the risk of estrogen-mediated endpoints, including an increase in the incidence of female breast cancer, interference with male reproductive functions and an influence on the

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Table 1
Measurement of vaginal opening (VO) day.

Chemical	Dose (mg/kg BW/day)		
	62.5	250	1000
VE		33.6 ± 3.23	
EE		21.4 ± 0.53[a]	
Methylparaben	34.4 ± 1.07	34.5 ± 1.96	36.8 ± 1.96[a]
Ethylparaben	35.0 ± 1.49	35.1 ± 1.52	34.7 ± 1.70
Propylparaben	33.0 ± 2.49	34.0 ± 1.33	34.6 ± 1.78
Isopropylparaben	35.2 ± 3.26	36.2 ± 1.03[a]	36.7 ± 0.71[a]
Butylparaben	34.1 ± 2.42	33.6 ± 1.35	34.1 ± 0.99
Isobutylparaben	31.6 ± 1.43	31.8 ± 1.62	33.6 ± 2.99

[a] $p < 0.05$ compared with VE.

development of malignant melanoma [17]. A linear relationship between parabens and cell proliferative potency, and parabens and ER-binding capacity, has been reported [18–20].

Little is known about the long-term health risks of paraben exposure during fetal or prepubertal stage. The effects become increasingly complex with respect to the age, reproductive history, and endocrine milieu of the host at the time of exposure. In rats, most spontaneous neoplasias (with the exception of leukemia) of the endocrine organs or of organs appear to be under endocrine control. It has been concluded that mechanism-based toxicology is not yet sufficient for human risk assessment, and this approach should be coupled to, and/or validated by, traditional long-term bioassays [21]. Thus, the elucidation of the molecular and biochemical mechanism(s) related to the potential effects of parabens in humans and animals is necessary. A few conventional short- and long-term systemic toxicity studies of parabens in animals treated orally are available, but there is insufficient information to demonstrate whether the adverse effects of parabens, seen in animals, are associated with estrogenic activity. As chemicals showing weak estrogenic activity [16], parabens can lead to a more general environmental estrogen problem. In animal uterotrophic assays, a reversible modification of the morphology and physiology of the uterus has been investigated [22–25]. In addition, paraben exposure has been shown to cause changes in global patterns of gene expression, to create aberrant estrogenic signaling in cells, to adversely influence breast cancer development [26,27], and to result in changes in the expression patterns of multiple genes in the rat fetal reproductive system [28,29]. Some previous studies have indicated that parabens are non-toxic, non-irritating and non-sensitizing to human and animals [26]. Parabens are quickly absorbed through the skin [30], they have been found in human breast tissue and human milk [31], and they have an effect on reproductive tissues, cardiovascular, skeletal and gastrointestinal systems. Parabens are thought to be converted into p-hydroxybenzoic acid by the hepatic metabolism, inferred from the detection of p-hydroxybenzoic acid as the main metabolite in the blood and urine of mammals exposed to parabens [32].

Despite the effects of parabens on human and animal health, these chemicals are officially approved food additives in many countries. The European Union permits the use of parabens at maximum concentrations of 0.4–0.8% in cosmetic products (EU Cosmetics Directive 76/768/EEC) and the daily intake is 0–10 mg/kg-day. In Japan, the daily intake permitted is 1.06 mg per person [33]. In the USA, the potential average daily intake is approximately 1–16 mg/kg for infants and 4–6 mg/kg for persons aged 2 years or older.

To date, there is no definitive data in the published literature from studies on the bioavailability, metabolism, short-term and long-term toxicity, reproductive toxicity, genotoxicity, and carcinogenicity of exogenous parabens administered orally. Reports on studies in experimental animal models on the mechanisms of parabens as estrogenic compounds are lacking. Further research is needed to provide new insights into the mechanism(s) through which endocrine disruptors elicit their effects on biological systems and on human and animal health. In this study, from the systemic evaluation of the paraben-induced effects on physiologi-

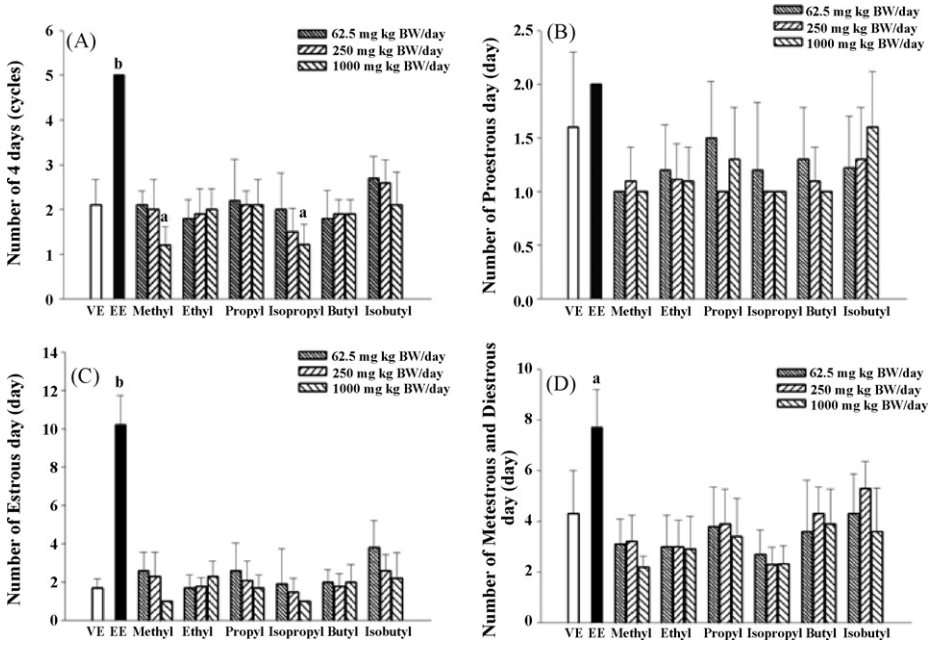

Fig. 1. Effects of parabens on estrous cycles. The estrous cycle of female rats ($n = 10$) following 3 weeks of dose-dependent (62.5, 250 and 1000 mg/kg BW/day) paraben exposure (methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutylparaben) via the oral route. The daily characterization of vaginal cytology in the rats is a noninvasive and reliable method for evaluating estrous cyclicity. Normalization of cycle length is 4-day cycle and separate into three distinct phases, commonly termed metestrus/diestrus, proestrus and estrus. To indicate toxicant-induced alterations in reproductive function, we evaluated the number of 4-day cycle and number of proestrus, estrus and metestrus/diestrus period during the period of chemical exposure. Rats treated with the high dose of methyl- and isopropylparaben showed changes in the 4-day estrus cycle ($p < 0.05$). Additionally, slight (but not statistically significant) changes were observed in the proestrous, estrous, metestrous, and diestrous stages of the estrous cycles. In contrast, a highly significant difference in both the number of 4-day cycles and the number of estrous days was found in the EE treated group. [a] $p < 0.05$; [b] $p < 0.01$, vs. control group.