Endocrine Disrupting Activities of Parabens: An Overview of Current Databases on Their Estrogenicity

Vu Hoang Dang and Eui-Bae Jeung*

Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea

ABSTRACT

Recently, parabens have been believed to act as xenoestrogens, an identified class of endocrine disruptors (EDs). These environmental compounds are the most well-known as preservatives in many commercial products, including food, cosmetics and pharmaceutical industries. It has been demonstrated that the human health risks of parabens result from a long-term exposure to skin in which this chemical group is rapidly absorbed through the skin. On the other hand, parabens are also completely absorbed from gastrointestinal tract. It has reported that these substances possess several biological effects in which inhibitory property involved in membrane transports and mitochondrial functions is considered to be important for their action. Testing of parabens has revealed that estrogen-like activities of these chemicals are much less potent than natural estrogen, 17β estradiol (E2). Additionally, the estrogenicity of individual paraben- compounds is distinct depending upon their biochemical structure. Recent findings of paraben-estrogenic activities have shown that these compounds may affect breast cancer incidence in women, suggesting adverse ecological outcomes of this environmental group on human and animal health. Although the biological and toxicological effects of parabens have been demonstrated in many previous studies, possible mechanism(s) of their action are required to be explored in order to bring the better understanding in the detrimental impacts of parabens in human and wildlife. There have several different types of parabens which are the most widely used as preservatives. These include methylparaben, ethylparaben, propylparaben, butylparaben and p-hydroxybenzoic acid, a major metabolite of parabens. In this review, we summarize current database based on *in vitro* and *in vivo* assays for estrogenic activities and health risk assessment of paraben- EDs which have been published previously.

(Key words : endocrine disruptors, estrogenicity, parabens)

INTRODUCTION

Estrogen (E2) plays an important role in many physiological processes and the development of various organs. This sex hormone has been identified as an essential factor in the control of the development of reproductive organs, bone, liver and cardiovascular systems (Watanabe *et al.*, 2003). On the other hand, E2 is crucial for the regulation of many pituitary hormones (Wilson *et al.*, 1998). Some reports have indicated the hormone-like activities of several environmental compounds, also called endocrine disruptors (EDs), which may alter the endocrine system(s) of human and wildlife via various mechanism(s). It has been also reported that some EDs, both synthetic and natural, can exert adverse effects on health, even at very low levels, through a variety of mechanisms i.e., binding to hormone receptors, mimicking hormones, or blocking the action of hormones (Dang *et al.*, 2007a; DeRosa *et al.*, 1998; McKinney and Waller 1998). Others may stimulate or inhibit enzymes that play important roles in hormone synthesis. The most well known class of EDs is xenoestrogens. These chemicals possess estrogen-like properties which may mimic the actions of physiological estrogen via estrogen receptors (Dang *et al.*, 2007b; Dang *et al.*, 2007c; Dang *et al.*, 2007d; Moggs 2005). Recently, synthetic estrogens have been used widely for the treatment of several diseases, including breast cancer and osteoporosis (Moggs 2005; Riggs and Hartmann 2003). However, the potential detrimental effects on growth and development of these drugs still remain to be elucidated. Also, there is increasing evidence that the interaction of these chemicals with hormone receptor systems may play a key role in ED-responses (Dang *et al.*, 2007c; Dang *et al.*, 2007d). The estrogenicity of EDs can be determined by measuring the ability of these com-

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* Correspondence : E-mail : ebjeung@chungbuk.ac.kr

pounds to bind to ERs and initiate a transcriptional response (McLachlan *et al.*, 2006). It has been reported that EDs may exert harmful effects on human health by altering hormonal balance or by disrupting the normal biological functions of several organ system(s), particularly during the critical development stage. In addition, EDs show distinct detrimental affects on humans and/or animals depending on their estrogenicity. While some EDs appear to be highly potent endocrine disruptors (Newbold *et al.*, 2004; Cupp and Skinner 2001), others have only weak estrogenic activity (Hong *et al.*, 2004a; Bolger *et al.*, 1998; Soto *et al.*, 1995). The estrogenic EDs, diethylstilbestrol (DES), phthalate acid ester, alkylphenols (APs), polychlorinated biphenyls (PCBs), phytoestrogens, and methothoxychlor are rapidly metabolized *in vivo* (Elsby *et al.*, 2001a; Elsby *et al.*, 2001b). It has been shown that phthalates, such as PCBs, DDT and its derivatives, certain insecticides and herbicides, such as Kepone and methoxychlor, plastic components, such as bis-phenol A (BPA), and detergents and their biodegradation products, such as alkylphenols, can bind to estrogen receptors (ERs) to induce or modulate the ER-mediated response (Choi and Lee 2004; Gray *et al.*, 1997; Laws *et al.*, 2000; Roy *et al.*, 1997). Some of these compounds, such as octyl-phenol (OP), nonyl-phenol (NP) and BPA, can easily pass through the maternal-placental barrier during pregnancy and adversely affect the normal function(s) of the neonatal reproductive system (Hong *et al.*, 2005, 2004a, 2004b 2003.). Recently, parabens were suggested to act as xenoestrogens, a class of endocrine disruptors (EDs). These compounds are widely used as preservatives in many commercial products, including food, cosmetics and pharmaceutical industries. The potency of paraben-EDs as estrogens has been shown to depend on the lengths of their alkyl side chains (Okubo *et al.*, 2001). An increase of alkyl group size may enhance paraben transactivation of ERs *in vitro* (Routledge *et al.*, 1998). Similar observations have been made for the alkyl phenol group, whose members are well-known as weak estrogenic agonists (Dang *et al.*, 2007d). It has been suggested that parabens, including methylparaben, ethylparaben, propylparaben, butylparaben and p-hydroxybenzoic acid, a major metabolite of parabens, exhibit potential estrogen-like characteristics. These chemicals have been shown to bind to the ER directly and modulate ER mediated responses. However, the ER binding ability and estrogenicity of these compounds are much less than those of E2 (Golden *et al.*, 2005; Routledge *et al.*, 1998). A recent study showed the presence of parabens in tissue samples from human breast tumors (Darbre *et al.*, 2004), suggesting a potential breast cancer risk of these preservatives. An *in vitro* experiment using MCF breast carcinoma cells has indicated that parabens, especially those with longer and branched alkyl side-chains, could induce cell proliferation (Okubo *et al.*, 2001). The induction of cell proliferation *in vitro* and *in vivo* by estrogens is a crucial event in the carcinogenesis of gynecological tissues (Dang *et al.*, 2007a; Bardin *et al.*, 2004). However, the detection of parabens in breast tumor tissues does not necessarily imply that they an environmental risk factor for human breast cancer. Further analysis and/or the identification of prognostic biomarkers for breast cancer are required to elucidate the carcinogenic effects of parabens.

To date, there is no perfect experimental model for examining the toxicological and biological effects of estrogenic EDs, including parabens, on humans and wildlife. In contrast, much experimental research has been expended to show the potential impacts of EDs using *in vitro* and/or *in vivo* models. Recently, biomarkers have been used widely to characterize the estrogenicity of EDs, in particular, at low doses. The biomarker response to EDs could be of crucial use for understanding the mode(s) of action of these compounds. Additionally, the measurement of biomarker gene response provides a very sensitive and powerful tool to identify estrogenic compounds in the environment (Choi and Jeung 2003). These include pS2, MUC1, androgen receptor, progesterone receptor, ER, clusterin, complement C3, lactoferrin, vitellogenin, cathepsin B (Ren *et al.*, 1997; Heppell *et al.*, 1995) and CaBP-9k (Dang *et al.*, 2007b; Dang *et al.*, 2007c; Dang *et al.*, 2007d; An *et al.*, 2003; Choi and Jeung 2003; An *et al.*, 2002).

In this review, we survey current databases containing information on the *in vitro* and *in vivo* estrogenicity of parabens as well previous publications dealing with the health risks associated with these compounds. The information provided here may be of importance when it becomes necessary to reconsider the use of parabens as preservatives in food, cosmetics and in the pharmaceutical industries. Furthermore, new research will be required to obtain new insights into the mechanism(s) through which EDs elicit their effects on biological systems and on human and animal health.

GENERAL DATABASE OF PARABENS

In this article, we review the various biological effects of parabens in the published literature. Parabens have multiple

biological effects, of which antimicrobial activity is the most important. The antimicrobial activities of pababens depend on the lengths of their alkyl groups (Golden *et al.*, 2005). Even at low concentrations, parabens are more effective against fungi than bacteria (Soni *et al.*, 2001). Previous studies have indicated that parabens are non-toxic, non irritating and non-sensitizing to human and animals (Prusakiewicz *et al.*, 2007). Parabens are quickly absorbed through the skin (Darbre *et al.*, 2004). Also, parabens are absorbed through the gastrointestinal tract. Once absorbed, they are hydrolysed to p-hydroxybenzoic acid, conjugated, and excreted in urine (Darbre *et al.*, 2004). The absorption of parabens through skin and subcutaneous fatty tissues may be affected by the presence of caboxylestase (Bando *et al.*, 1997), and consequently this may influence their hydrolysis to p-hydroxybenzoic acid (Lobemeier *et al.*, 1996). Parabens have been approved by the United States Food and Drug Administration as preservatives in commercial products (Prusakiewicz *et al.*, 2007). However, recent work has shown that parabens possess estrogen-like activities. For this reason they have been termed xenoestrogens, a well-known class of EDs.

Xenoestrogens can cause biological and toxicological effects by altering the endocrine system in many ways. Importantly, these estrogenic EDs can bind to ERs and modulate physiological responses to estrogen. However, the mechanism via which xenoestrogens exert their adverse effects on the body remains unclear. In addition, EDs differ greatly in their detrimental affects on humans and/or animals and this depends on their estrogenicity. Alky phenols are branched molecules with typically octyl-, nonyl-, or dodecyl- chains that create a variety of isomers mostly at the para-position of the phenolic ring (Dang *et al.*, 2007a; Hong *et al.*, 2004a). In addition, alkylphenols display rapid conjugation and excretion, particularly when the rodent are exposed by the oral route (Upmeier *et al.*, 1999; Certa *et al.*, 1996). Also, the lipophilic properties of these chemicals allow them to accumulate in fatty tissues of human and animals (Darbre *et al.*, 2003; Turusov *et al.*, 2002; Stellman *et al.*, 1998). Like all xenoestrogens, parabens can mimic the effects of the physiological estrogen, E2. They may bind to ERs, stimulate the ER-dependent response, and/or influence the expression of estrogen-responsive genes, including ER α, a progesterone receptor (PR), and pS2. *In vitro* studies have indicated that the binding affinity of parabens for ER α also depends upon the length of the alkyl chain (Byford *et al.*, 2002). Additionally, the results of competitive binding assays indicate that these compounds are at least 10,000 to 100,000 times less potent than E2. An increase in cell growth was also observed following paraben exposure of MCF human breast cancer cell lines (Byford *et al.*, 2002; Darbre *et al.*, 2002). The effects of parabens on cell proliferation were inhibited by co-treatment with ICI 182,780, suggesting a potential role for ERs in the cellular response to EDs. Since the first study conducted by Routledge (Routledge *et al.*, 1998), a variety of *in vivo* bioassays have been developed to elucidate the mechanism(s) underlying the effects of ED-exposure on various mammals, including rodent uterus.

The uterus of rodent is considered to be an ideal organ for screening the estrogenicity of EDs. Based on the increase in the uterine weight of immature rodents after exposure to environmental EDs, an uterotrophic assay was considered to be the most reliable *in vivo* test for the detection of estrogenic agonists and antagonists (Dang *et al.*, 2007e; Owens and Ashby 2002). A previous report using immature and ovariectimized mice indicated that p-hydroxybenzoic acid (PHBA), the major metabolite of parabens, can increase the cornification of vaginal epithelial cells and elicit uterotrophic activities in a dose dependent manner. Compared with E2 (1 μg/100 g BW), the uterotrophic response caused by 500 μg/100 g BW of PHBA was 0.0011 for immature mice and 0.0018 for ovariectomized mice (Lemini *et al.*, 1997). Other experiments conducted by Routledge *et al.* (1998) also showed an increase in uterine wet and dry weights in immature rats exposed to parabens. However, the uterus weights were not normalized to body weights (Golden *et al.*, 2005). In contrast to the positive uterotrophic response reported by Lemini (1997), PHBA failed to induce an estrogen-like activity in the mouse uterotrophic bioassay (Golden *et al.*, 2005; Hossaini *et al.*, 2000). The terms false negative and false positive in uterotrophic bioassays were mentioned previously in work where very weak agonists generated a false negative result and false positives were generated with negative chemicals (Owens and Ashby 2002). Also, certain well-known non-estrogen substances may cause a positive uterotrophic response (Nelson *et al.*, 1991; Gardner *et al.*, 1989; Mukku and Stancel 1985; Jones and Edgren 1973). Further experiments in animals are necessary to validate the positive findings reported by Lemini (Golden *et al.*, 2005). One of the molecular events accompanying the increased uterine weight evoked by estrogenic ED-action was shown to be the transcription of ER-mediated genes in the rodent uterus (Owens and Ashby 2002; Bolger *et al.*, 1998; Gould *et al.*, 1998; Danzo 1997; Gaido *et al.*, 1997; Bulger *et al.*, 1978). However, our knowledge of the estrogenicity

of parabens *in vitro* and *in vivo* is still very limited. Recent critical evidence has shown that several parabens accumulate in breast tumor tissue, indicating the potential risk of these estrogenic EDs for breast cancer. Further studies are required to elucidate the molecular and biochemical mechanism(s) underlying the possible harmful effects of paraben exposure in human and animals, particularly during the critical developmental stages in these organisms.

ESTROGENICITY OF PARABENS

1. *p*-Hydroxybenzoic Acid (PHBA)

PHBA, the main metabolite of parabens, is derived from benzoic acid, a compound with antimicrobial activities. PHBA is commonly used as a preservative in food, cosmetic and pharmaceutical formulations. PHBA was shown previously to be a weak estrogen. This was based on work done by Allen and Doisy using vaginal cornification of ovariectomized adult mice as a model and on work done by Evans who measured the uterotrophic effect of this compound in immature female mice (Lemini *et al.*, 1997). Lemini *et al.* examined the estrogenic activity of PHBA in CD1 mice by exposing them for 3 days to PHBA delivered by subcutaneous injection. There results showed that PHBA elicited an estrogenic response in a dose dependent manner. Vaginal cornification and uterotrophic activities were seen in both immature and adult ovariectomized mice at a dose of 5 mg/kg BW/day. In addition, the potency of this chemical was evaluated to be approximately 1,000 times less than that of E2. However this positive effect was not confirmed and/or validated by Hossaini (Hossaini *et al.*, 2000). They reported that treatment with PHBA failed to induce an estrogenic response in the mouse uterotrophic bioassay, in which estradiol benzoate, a positive control, significantly enhanced the weight of the mouse uteri (Hossaini *et al.*, 2000). Similar results also obtained with the rat uterotrophic assay in which no estrogenic effects were noted following *in vivo* administration of PHBA at a dose of 5 mg/kg BW. However, the body weight gain was significantly reduced in the group treated with PHBA and/or in the positive control group when compared to the vehicle control group. *In vitro* assays also indicated that PHBA was inactive and unable to compete with ^{3}H- estradiol for binding to rat ERs (Hossaini *et al.*, 2000; Routledge *et al.*, 1998).

2. *p*-Hydroxybenzoic Acid Methyl Ester (Methylparaben)

Methylparaben is an ester of PHBA. This chemical has a long history of use in foods, drugs and cosmetics as an antimicrobial preservative (Soni *et al.*, 2002). In an *in vitro* yeast based estrogen assay, methylparaben was reported to be weakly estrogenic (Routledge *et al.*, 1998), and the estrogenic activity of this chemical was inhibited by co-exposure to 4-hydroxy tamoxifen, a well-known antiestrogen, suggesting an interaction between this paraben and the ER system in yeast cells. In addition, the estrogenicity of methylparaben has also been demonstrated in an MCF human breast cancer cell line by Byford *et al.* (Byford *et al.*, 2002). An increase in both the expression of estrogen-responsive genes and cell proliferation was reported. For instance, methylparaben at a concentration of 10^{-4}M increased the expression of the pS2 gene. The proliferation effects evoked by this ED were completely inhibited when it was co-administrated with a pure ER antagonist, ICI 182,780. The *in vivo* estrogenic activity of methylparaben has been tested using the rodent uterotrophic bioassay, in which it failed to increase uterus weight. Oral treatment with 800 mg/kg BW of methylparaben did not increase uterine weight in immature rats. Similar results were also obtained following subcutaneous administration of 80 mg/kg BW/day in this model (Golden *et al.*, 2005; Routledge *et al.*, 1998). Also, no mouse uterotrophic activity was reported following gavage with methylparaben at a dose up to 100 mg/kg BW/day (Hossaini *et al.*, 2000). A recent study showed that parabens can be detected in human breast cancer tissues in which the highest level of methylparaben was reported, suggesting that this chemical accumulates from the environment in human body tissue Additionally, sixty- two percent of all extracted parabens is methylparaben, reflecting its widespread use in consumer products (Daston 2004; Rastogi *et al.*, 1995).

3. *p*-Hydroxybenzoic Acid Eethyl Ester (Ethylparaben)

Ethylparaben is an identical class of parabens. Like all parabens, ethylparaben possesses estrogen-like activity in a yeast-based estrogen assay and in a yeast two-hybrid assay (Okubo *et al.*, 2001). The estrogenic potency of ethylparaben is about 150,000 times lower than that of E2. Also, this ED exhibits an effect on cell proliferation, in which maximal proliferation was observed at a concentration of 2×10^{-5}M (Okubo *et al.*, 2001). In an MCF7 human breast cancer cell line, ethylparaben induces an increase in the expression of both transfected (ERE-CAT reporter gene) and endogenous (pS2) estrogen-regulated genes. Furthermore, this ED increased cell proliferation in momolayer cultures. Interestingly, the proliferation effect of ethylparaben was inhibited by the pure ER antagonist, ICI

128,780 indicating the crucial mediatory role of ERs in its effect on cell proliferation. Although *in vivo* assays have been developed to examine the possible effects of ethylparaben, a lot still remains to be done concerning the estrogenicity of ethylparaben. Padersen *et al.* (2000) has reported that ethylparaben is about 6 time less active than butylparaben for the induction of the estrogen response *in vivo*. Another study conducted by Hossaini *et al.* (2000) showed that ethylparaben does not induce the uterotrophic response. Importantly, this ED was identified in human breast cancer tissue by thin layer chromatography, suggesting that it may have an impact on human health (Daston 2004).

4. *p*-Hydroxybenzoic Acid Butyl Ester (Butylparaben)

A variety of *in vitro* and *in vivo* studies have focused on the biological and toxicological effects of butylparaben, a commonly used preservative. Butylparaben has weak estrogenic activity and low affinity for the ER *in vitro* (Daston 2004; Routledge *et al.*, 1998). In competitive binding assays, this chemical was approximately five orders of magnitude less active than DES, a potent synthetic estrogen. Also the effects of butylparaben were inhibited by co-administration of 4-hydroxy tamoxifen, indicating that an interaction between this compound and the ER system is required to induce the estrogenic response. Additionally, butylparaben stimulates cell proliferation, and this effect can be blocked in MCF7 cell lines by ICI 128,780, an antiestrogen. Several biomarker genes, such as pS2, ER alpha or PR, have been used to examine the estrogenic activity of butylparaben. A down-regulation in ER alpha expression and an up-an regulation of PR levels following ED-exposure were noted in human breast cancer MCF7 cells (Okubo *et al.*, 2001). Furthermore, the expression of pS2, a well-known estrogen-responsive gene, was also affected by ED treatment in this cell line (Byford *et al.*, 2002). In *in vivo* experiments, the results of a rat uterotrophic bioassay showed that subcutaneous administration of this chemical at a dose of 600 mg/kg BW had a positive effect on uterus weight (Hossaini *et al.*, 2000; Routledge *et al.*, 1998). In addition, modest increases in uteus wet and dry weights were observed when immature rats were orally administered doses ranging from 800 to 1,200 mg/kg BW/day. In extracts of human breast cancer tissue, butylparaben represented 11% of the total paraben content (Darbre *et al.*, 2004).

5. *p*-Hydroxybenzoic Acid Propyl Ester (Propylparaben)

Propylparaben has been recognized as an antimicrobial additive for over 50 years. It is most commonly used for the preservation of cosmetics (Soni *et al.*, 2002; Soni *et al.*, 2001; Gruvberger *et al.*, 1998). Previous studies reported that can it can easily be absorbed via the gastrointestinal tract and dermis (Soni *et al.*, 2001). After examining the bioaccumulation of this paraben and other parabens in human breast cancer tissue, Darbre *et al.*, found a high level of propylparaben (mean value of 2.6±0.9 ng g^{-1} tissue) (Darbre *et al.*, 2004), suggesting that parabens in commercial products, including cosmetics, food and drugs, are retained in human body tissues without hydrolysis. These findings are in agreement with previously reported data in which the estrogen-like activities of parabens in cultured human breast cancer cells were found to be due to the esters themselves and not to a common metabolite (Darbre *et al.*, 2003; Darbre *et al.*, 2002; Byford *et al.*, 2002). Also the estrogenic effects of propylparaben have been demonstrated in a number of *in vitro* studies. In the yeast-based estrogen assay, propylparaben was found to have weak estrogenic activity. The estrogenic potency of this ED was evaluated to be equivalent to that of 4-nonyphenol (Routledge *et al.*, 1998). In addition, this compound induced cell proliferation in MCF7 human breast cancer cell lines, in which maximal proliferation was observed at 2×10^{-5}M propylparaben (Okubo *et al.*, 2001). The pS2 gene has been used as a biomarker for screening the estrogenic activities of EDs in many previous studies. In MCF7 cells, the highest level of pS2 expression was observed when this chemical was compared to other chemicals and/or the control (Byford *et al.*, 2002). The uterotrophic bioassay also showed that this chemical fails to induce the growth of mouse uteri at a dose of 100 mg/kg BW (Hossaini *et al.*, 2000). Further analysis is needed to acquire a better understanding of the estrogenic effects of propylparaben *in vivo*.

6. *p*-Hydroxybenzoic Acid Isobutyl Ester (Isobutylparaben)

On the basis of the resemblance of its chemical structure to physiological estrogen, E2, isobutylparaben has been suggested to bind to ERs and cause dysregulation of estrogen-responsive gene expression (Darbre *et al.*, 2002; Blair *et al.*, 2000). This ED has lipophilic properties and antimicrobial activities (Golden *et al.*, 2005), and has estrogenic activity in *in vitro* and *in vivo* assays. It is able to displace [^{3}H] estradiol from cytosolic ERα on MCF7 human breast cancer cells. In these assays, [^{3}H] estradiol displacement by isobutylparaben was 81% when it was present at a 10^5-fold molar excess over that of estradiol. Moreover, exposure to isobutylparaben at a concentrations of

10^{-5}M increased CAT gene expression in a MCF7 cell line stably transfected with the estrogen-responsive ERE-CAT reporter gene (Darbre *et al.*, 2002). Interestingly, a previous study reported induction of pS2 expression, a well established estrogen-regulated gene, by parabens, including methylparaben, ethylparaben, butylparaben and propylparaben (Byford *et al.*, 2002). These results are in agreement with recent findings on the estrogenicity of isobutylparaben, which showed that it can increase the expression of the pS2 gene in MCF7 cells at a concentration of 10^{-5}M (Darbre *et al.*, 2002). Okubo *et al.* examined the effects of this ED on the induction of other biomarker genes, and found that exposure to isobutylparaben could evoke a decrease in the expression level of ER α and an increase in the transcriptional level of PR in MCF7 cells (Okubo *et al.*, 2001). In order to examine the effects of isobutylparaben in cell proliferation, Darbre indicating that this ED could cause a proliferation response in two human breast cancer cell lines, MCF7 and ZR-75-1. Furthermore, these proliferation effects were inhibited by co-exposure to ICI 128,780, an antiestrogen (Darbre *et al.*, 2002). Like all parabens, isobutylparaben also possesses *in vivo* estrogenic activity as shown by its ability to cause uterus growth of uterus in a mouse uterotrophic bioassay. Darbe reported the bioaccumulation of this chemical in the human breast area and suggested that it could potentially influence the accidence and treatment of breast cancer (Darbre *et al.*, 2004).

7. *p*-Hydroxybenzoic Acid Isopropyl Ester (Isopropylparaben)

Okubo *et al.* indicated that isopropylparaben could induce cell proliferation effects with the highest response being observed at 5×10^{-6}M in MCF7 cells. The proliferative potency of isopropylparaben is about 170,000 folds lower than that of E2 (Okubo *et al.*, 2001). Additionally, the results of competitive binding assays with human ER α and ER β *in vitro* have revealed that the relative binding affinities of parabens, including isopropylparaben, were much lower than that of DES for these receptors. However, critical information about the toxic endpoints of this parabens is still not forthcoming (Harvey and Darbre 2004). Further assessment of the biological and toxicological effects of isopropylparaben *in vitro* and *in vivo* is required.

CONCLUSION

Information about the detrimental effects of parabens is very important for predicting the adverse effects of these compounds on humans and wildlife. Additionally, this information will be crucial when it comes to revising current strategies for the use of parabens as preservatives in food, cosmetics and the pharmaceutical industry. However, the information in current databases about the hormonal activities of these EDs is still very limited. Furthermore, there is no currently valid biomarker system or global genomic approach for evaluating the estrogenicity of parabens. More experiment research, both in *in vivo* and *in vitro,* is required to provide new insights into the how these EDs interfere with biological systems and exert their harmful effects on human and animal health.

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