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Alkyl Hydroxy Benzoate Preservatives (Parabens) Are Estrogenic Compounds; Their Adverse Effects on Animals and Human

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I. INTRODUCTION

It has recently been suggested that the release of "endocrine disrupters (EDs)" into the environment has resulted in adverse health effects on wild life populations and humans (Golden *et al.*, 1998; Tyler *et al.*, 1998; Kang *et al.*, 2000). Human sperm counts have declined significantly throughout the world during the past fifty years, and which is a significant public health concern (Carlsen *et al.*, 1992; Carlsen *et al.*, 1995). In addition, the EDs persisting in the environment are known to disrupt the normal endocrine systems of wildlife (Colborn, 1995; Crew *et al.*, 1995; Folmer *et al.*, 1996; Sumpter, 1995; Tyler, 1998). Some estrogenic chemicals bind to estrogen receptors (Bolger *et al.*, 1998), interfere with the binding of physiological ligands to steroid hormone-binding proteins (Danjo, 1997; Milligan *et al.*, 1998), and show immunotoxicity (Sakae *et al.*, 1998). Such estrogenic compounds, structurally heterogeneous and nonsteroidal, were found in many places including the environment (Giger *et al.*, 1984; Jobling *et al.*, 1995; White *et al.*, 1994), food cans (Brotons *et al.*, 1995), experimental tools (Krishnan *et al.*, 1993; Soto *et al.*, 1991), dental sealant (Olea *et al.*, 1996), pharmaceuticals (Harnagea-Theophilus *et al.*, 1998), and cosmetics (Routledge *et al.*, 1998).

Nowaday, man-made or natural chemicals (environmental estrogens) were debated that they could be linked to a number of human reproductive deficits. These deficits include decreased sperm production and increased incidence of hypospadias and cryptorchidism in human population (Sharpe and Skakkebæk, 1993; Toppari *et al.*, 1996). It is now realized that certain synthetic compounds, used in a wide range of products, can mimic the effects of the main natural estrogen, 17β-estradiol, by binding to estrogen receptor and influencing the expression of estrogen-dependent genes (Park *et al.*, 2000; Routledge *et al.*, 1998). This realization has coincided with epidemiological data suggesting, a progressive decline in human male reproductive health and fertility (Carlsen *et al.*, 1992, 1995).

p-Alkylphenols, breakdown products of surfactants, detergents, toiletries, and herbicides, have been found in sewage effluent, and are environmental estrogen. They competitively bind to the estrogenic receptor, enhance mammalian breast cancer cell proliferation, and are transcriptionally active (Muller and Kim, 1978).

Alkylphenols produce an estrogenic response in fish and rats similar to that elicited by estradiol, but those chemicals are 1000 times less potent than estradiol (Bicknell *et al.*, 1995).

II. PARABENS ARE TOILETRIES, COSMETICS, PHARMACEUTICAL PRODUCT COMPONENTS AS A PRESERVATIVES

Parabens, a compound with anti-bacterial and anti-fungal properties, were extensively used as food preservatives and cosmetics. In 1981, the Food and Drug Administration reported the use of methyl-, ethyl-, propyl, and butyl paraben in over 13,200 formulations (Elder, 1984). The popular use of paraben preservatives in cosmetics and toiletries arises from their low toxicity, inertness, broad spectrum of activity, worldwide legislative acceptance, biodegradability, and cost (inexpensive), also because they are considered to be

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safe (Elder, 1984).

The average daily intake of parabens in foods is estimated to be 1~16 mg/kg for infants and 4~6 mg/kg for persons aged 2 years or older. Maximun levels of parabens in pharmaceutical products seldom exceed 1% w/w, and the EEC directives 76/768/EEC and the Danish cosmetic regulations permit the presevation of cosmetic products with MP, EP, PP and BP up to a maxium combined concentration of 0.8% w/w.

Parabens preserve fats, proteins, oils and gums in hair shampoos and conditioners, skin care products (cleansers and moisturisers), colognes, perfumes, toothpastes, soaps, and a range of make-up and sun-care products.

In a recent survey of 215 cosmetic products, 77% of the rinse-off products and 99% of the leave-on products contained parabens. Therefore, products containing parabens may be applied to the skin, hair, scalp, lips, mucosae (oral, occular, vaginal), and nails, either ocassionally or on a daily basis, and their use may extend over a period of year.

III. THE ESTROGENIC EFFECT OF PATABENS IN RECOMBINANT YEAST SYSTEM

Parabens were structurally similar to alkylphenols which are known to be weakly estrogenic (Soto *et al.*, 1991; Routledge *et al.*, 1997, 1998; Hossaini *et al.*, 2000) (Fig. 1).

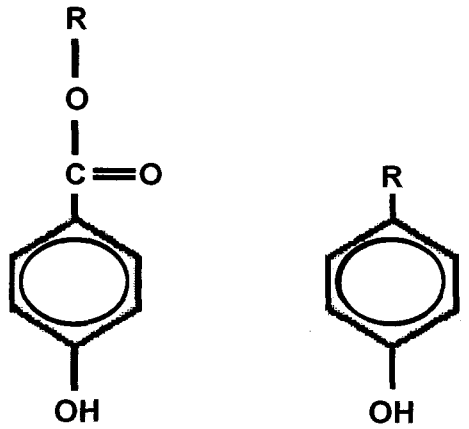

Fig. 1. Structural Similarity of Parabens and Alkylphenolic Chemicals.
R=CH_3 (A = methylparaeben; B = methylphenol)
R=C_2H_5 (A = ethylparaeben; B = ethylphenol)
R=C_3H_7 (A = propylparaeben; B = propylphenol)
R=C_4H_9 (A = butylparaeben; B = butylphenol)

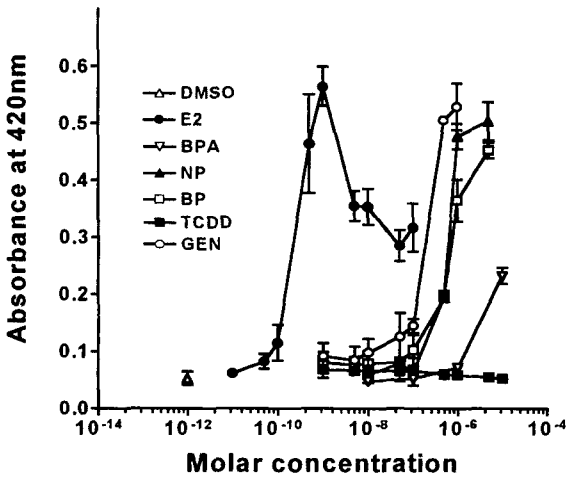

Fig. 2. Estrogenic activity of butyl paraben in the yeast containing human estrogen receptor and β-galatosidase reporter gene.

Routledge *et al.* (1998) reported that four most widely used parabens (namely methyl-, ethyl-, propyl-, and butylparaben) were all found to be weakly estrogenic and the most potent compound was butyl p-hydroxybenzoic acid (butylparaben, BP) using *in vitro* yeast-based estrogen assay (Park *et al.*, 2000).

IV. THE ESTROGENIC EFFECTS OF PARABENS IN THE ANIMALS

Several papers reported that parabens were to have estrogenic properties using *in vivo* and *in vitro* systems (Lemini *et al.*, 1997; Routledge *et al.*, 1998; Hossani *et al.*, 2000). Four days after paraben (0.5, 5, 50, and 500 μg/100 g) treatment produced a dose-dependent response on vaginal cornification and uterotrophic activity in both immature and adult ovariectomized mice (Lemini *et al.*, 1997). In a receptor-binding assay, BP was able to compete with ^{3}H-estradiol for binding to the rat estrogen receptor with an affinity approximately 5 orders of magnitude lower than that of diethylstilbestrol, and between 1 and 2 orders of magnitude less than nonylphenol. In an *in vitro* yeast-based estrogen assay, the four most widely used parabens (namely methyl-, ethyl-, propyl-, and butylparaben) were all found to be weakly estrogenic with the most potent (butylparaben) being 10,000-fold less potent than 17β-estradiol. Also, subcutaneous administration of butylparaben produced a posi-

tive uterotrophic response *in vivo*, although it was approximately 100,000 times less potent than 17β-estradiol (Routledge *et al.*, 1998). In immature Wistar rats, subcutaneous administration of butylparaben produced a weak estrogenic response at 600 μg/kg body weight per day (Hossani *et al.*, 2000).

V. CONCLUSION

Parabens are widely used in the nutrition and cosmetic industry, a broad range of human population could be exposed to BP. The existence of hormonal activity in chemical agents widely and constantly used that can act as "endocrine disrupters" is a matter of concern for the possible impact on reproductive health in next generations in human beings and wildlife populations. Therefore, we hope that the substitute materials of parabens will be come out in near future. However, what problem we confront is that we don't have any substitution of parabens like bisphenol A, even if there are varieties of usage of these chemicals in human life, now.

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REFERENCES

Bicknell, R.J., Herbison, A.E. and Sumpter, J.P. (1995): Oestrogenic activity of an environmentally persistent alkylphenol in the reproductive tract but not the brain of rodents. *J. Steroid. Biochem. Mol. Biol.*, **54**, 7-9.

Bolger, R., Wiese, T.E., Ervin, K., Nestich, S. and Checovich, W. (1998): Rapid screening of environmental chemicals for estrogen receptor binding capacity. *Environ. Health. Perspect.*, **106**, 551-557.

Brotons, J.A., Olea-Serrano, M.F., Villalobos, M., Pedraza, V. and Olea, N. (1995): Xenoestrogen released from lacquer coatings in food cans. *Environ. Health. Perspect.*, **103**, 608-612.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N.E. (1992): Evidence for decreasing quality of semen during past 50 years. *Br. Med. J.*, **305**, 609-613

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N.E. (1995): Declining semen quality and increasing incidence of testicular cancer: Is there a common cause? *Environ. Health. Perspect.*, **103**(Suppl7), 137-139.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebæk, N.E. (1992): Evidence for decreasing quality of semen during past 50 years. *Br. Med. J.*, **305**, 609-613.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebæk, N.E. (1995): Declining semen quality and increasing incidence of testicular cancer: Is there a common cause? *Environ. Health Perspect.*, **103**(Suppl 7), 137-139.

Colborn, T. (1995): Environmental estrogens: Health implications for human and wildlife. *Environ. Health. Perspect.*, **103**, 147-150.

Crew D., Bergeron, J.M. and McLachlan, J.A. (1995): The role of estrogen in turtle sex determination and the effect of PCBs. *Environ. Health. Perspect.*, **103**(suppl7), 73-77.

Danjo, B.J. (1997): Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. *Environ. Health. Perspect.*, **105**, 294-301.

Elder, R.L. (1984): Final report on the safety assessment of methylparaben, ethylparaben, propylparaben, and butylparaben. *J. Am. Coll. Toxicol.*, **3**, 147-209.

Folmer, L.C., Denslow, N.D., Rao, V., Chow, M., Crain, D.A., Enblom, J., Marcino, J. and Guilette, L.J. Jr. (1996): Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (Cyprinus carpio) captured near a major metropolitan sewage treatment plant. *Environ. Health. Perspect.*, **104**, 1096-1101.

Giger, W., Brunner P.H. and Schaffner, C. (1984): 4-Nonylphenol in sewage sludge: Accumulation of toxic metabolites from nonionic surfactants. *Science.*, **225**, 623-625.

Golden, R.J., Noller, K.L., Titus-Ernstoff, L., Koufman, R.H., Mittendorf, R., Stillman, R. and Reese, E.A. (1998): Envrionmental endocrine modulators and human health: An assessment of the biological evidence. *Crit. Rev. Toxicol.*, **28**,109-227

Harnagea-Theophilus, E., Gadd, S.L., Knight-Trent, A.H., DeGeorge, G.L. and Miller, M.R. (1998): Acetaminophen-induced proliferation of breast cancer cells involves estrogen receptors. *Toxicol. Appl. Pharmacol.*, **155**, 273-279.

Hossaini, A., Larsen, J. and Larsen, J.C. (2000): Lack of oestrogenic effects of food preservatives (parabens) in uterotrophic assays. *Food Chem. Toxicol.*, **38**, 319-323.

Jobling, S., Reynolds, T., White, R., Parker, M.G. and Sumpter, J.P. (1995): A variety of environmentally persistent chemicals, including some phthalate plasticiz-

ers, are weakly estrogenic. *Environ. Health. Perspect.*, **103**, 582-587.

Kang, K.S., Li, G.X., Park, J.S., Lee, B.J., Che, J.H., Tai, J.H., Cho, J.J., Kim, S.H., Lee, D.S. and Lee, Y.S. (2000): Effect of green tea on prostate and seminal vesicle in rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. of Microbiol. Biotechnol.*, **10**(3), 281-286.

Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L. and Feldman. D. (1993): Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology.*, **132**, 2279-2286.

Lemini, C., Silva, G., Timossi, C., Luque, D., Valverde, A., González-Martínez, M., Hernández, A., Rubio-Póo, C., Chávez Lara, B. and Valenzuela, F. (1997): Estrogenic effects of *p*-hydroxybenzoic acid in CD1 mice. *Environmental Research*, **75**, 130-134.

Milligan, S.R., Khan, O. and Nash, M. (1998): Competitive binding of xenobiotic oestrogens to rat alpha-fetoprotein and to sex steroid binding proteins in human and rainbow trout (Oncorhynchus mykiss) plasma. *Gen. Com. Endocrinol.*, **112**, 89-95.

Muller, G.C. and Kim, U.-H. (1978): Displacement of estradiol from estrogen receptors by simple alkylphenols. *Endocrinology*, **102**, 1429-1435.

Olea, N., Pulgar, R., Pedez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M. and Sonnenschein, C. (1996): Estrogenicity of resin-based composites and sealants used in dentistry. *Environ. Health. Perspect.*, **104**, 298-305.

ParK, J.-S., Lee, B.-J., Kang, K.-S., Tai, J.-H., Cho, J.-J., Cho, M.-H., Inoue, T. and Lee, Y.-S. (2000): Hormonal effects of several chemicals in recombinant yeast, MCF-7 cells and uterotrophic assay in mice. *J. of Microbiol. Biotechnol*, **10**(3), 293-299.

Routledge E.J., Odum, J.P., Ashby, J. and Sumpter, J.P. (1998): Some alkyl hydroxy benzoate preservatives (Parabens) are estrogenic. *Toxicol Appl Pharmacol.*, **153**, 12-19.

Routledge, E.J., Parker, J., Odum, J., Ashby, J. and Sumpter, J.P. (1998). Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Appl. Pharmacol.*, **153**, 12-19.

Sakae, K., Okuma, M., Kazuno, M., Yamaguchi, T., Yoshida, T., Furuya, H., Kayama, F., Suwa, Y., Fujii, W. and Fresa, K.L. (1998): Estrogenic xenobiotics affect the intracellular activation signal in mitogen-induced human peripheral blood lymphocytes: immunotoxicological impact. *Int. J. Immunopharmacol.*, **20**, 205-212.

Sharpe, R.M. and Skakkebæk, N.E. (1993): Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet*, **341**, 1392-1395.

Soto, A.M., Justica, H., Wray J.W. and Sonnenschein, C. (1991): ρ-Nonylphenol: An estrogenic xenobiotic released from modified polystyrene. *Environ. Health. Perspect.*, **92**, 167-173.

Soto, A.M., Justica, H., Wray, J.E. and Sonnenschein, C. (1991): P-nonylphenol: An estrogenic xenobiotic released from modified polystyrene. *Environ. Health Perspect.*, **92**, 167-173.

Sumpter, J.P. (1995): Feminized responses in fish to enviromental estrogen. *Toxicol. Lett.*, **82-83**, 737-742

Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L.J., Jégou, B., Jensen, T.K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J.A., Meyer, O., Müller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J. and Skakkebæk, N.E. (1996): Male reproductive health and environmental xenoestrogens. *Environ. Health Perspect.*, **104**(Suppl4), 741-803.

Tyler, C.R, Jobling, S. and Sumpter J.P. (1998): Endocrine disruption in wild life: A critical review of the evidence. *Crit. Rev. Toxicol.*, **28**, 319-361.

Tyler, C.R, Jobling, S. and Sumpter J.P. (1998): Endocrine disruption in wild life: A critical review of the evidence. *Crit. Rev. Toxicol.*, **28**, 319-361.

White, R., Jobling, S., Hoarse, S.A., Sumpter, J.P. and Parker, M.G. (1994): Environmental persistent alkylphenolic compounds are estrogenic. *Endocrinology.*, **135**, 175-182.