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Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use.

DRAFT

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1. Introduction

The European Commission has decided to revise the “Guideline on excipients in the label and package leaflet of medicinal products for human use (CPMP/463/00 Rev.1)” and a concept paper on the need for such revision has been published in 2012 (EMA/CHMP/SWP/888239/2011). Parabens used in medicinal products is one of the priorities among excipients under revision.

Parabens are currently listed in the guideline on excipients in the label and package leaflet of medicinal products for human use (CPMP/463/00 Rev.1) for their allergic potential. Further safety concerns have been raised during the last decade by the scientific community, regulatory agencies and the general public as a consequence of possible endocrine-disrupting effects (Darbre et al, 2004).

Parahydroxybenzoate esters and their sodium salts, usually named parabens, have been used for many decades as antimicrobial preservative in cosmetics, food products and pharmaceutical formulations. Parabens are effective over a wide pH range with a broad spectrum of antimicrobial activity, and are also effective against yeasts and molds. Antimicrobial activity increases with increasing alkyl chain length for the commonly used methyl, ethyl, propyl, and butyl parabens, and synergy between parabens has been reported (Charnock and Finsrud, 2007). In oral pharmaceutical formulations, combinations of methylparaben and propylparaben are applied with concentrations generally ranging from 0.015 to 0.2% for methylparaben and 0.02% to 0.06% for propylparaben (Rowe et al, 2006). Other parabens are also used in pharmaceuticals to a lesser extent, such as ethylparaben and butylparaben. The latter is predominantly used in pharmaceutical formulations for the cutaneous route.

The current reflection paper addresses methyl- and propylparaben, as those are the parabens predominantly used in oral pharmaceutical formulations. Given the public concerns referred to above, the focus of this document is on possible endocrine-disrupting effects in humans.

After finalisation of this reflection paper, the EMA may propose an updated wording of parabens in the next revision of the “Guideline on Excipients in the Label and Package Leaflet of Medicinal Products for Human Use (CPMP/463/00).

2. Discussion

2.1. Absorption, Distribution, Metabolism and Excretion (ADME)

Animal data

In a recent study (Aubert et al, 2012), the absorption (plasma area under the curve) of methylparaben and propylparaben was studied following a single oral administration to the rat at a dosage of 100 mg/kg. Following oral administration, the two substances showed a peak concentration in the blood between 30 min and 1 h post-dosing and the absorption was shown to be almost complete (88 to 95%). The absorption was higher in females than males. Moreover, the absorption was shown to be dependent upon the length of the paraben ester chain, thus the relative absorption of propylparaben constituted 83% of the absorption of methylparaben.

In addition, the ADME profile of parabens has been determined in rats, dogs and rabbits (Jones et al, 1956, Tsukamoto and Terada, 1964, Kiwada et al, 1980). Parabens appear to be very rapidly metabolized since only negligible levels of the parent compounds are detected in the blood within minutes after oral administration and paraben metabolites can be detected in the urine within an hour post-dosing. Irrespective of the species studied, the metabolism of parabens resulted in hydrolysis to

the principal metabolite para-hydroxybenzoic acid (PHBA). PHBA may be conjugated with glycine, glucuronic acid and sulfate to form para-hydroxyhippuric acid, PHBA-glucuronide or para-carboxyphenyl sulfate. Excretion is principally urinary and fast with more than 90% of the dose excreted within 24 h post-dosing.

Human data

The few human oral studies available generated similar results to those of the laboratory animal studies (Jones et al, 1956). Oral administration of methylparaben gives rise to fast absorption and marked and fast metabolism. The parent compound is found at negligible levels in the blood and PHBA is detected 3 minutes post-dosing. PHBA predominates among the urinary metabolites where it constitutes more than 50% of the administered dose 12 h after dosing. Data from biomonitoring studies showed (summary in SCCS/1446/11) that only small proportions of free parabens were detected whereas conjugates of parabens consisting of glucuronides and sulfate esters predominated both in serum and in urinary samples of adults. Higher proportions of free parabens were determined in urinary spot samples from preterm infants compared to adults.

Various types of esterases are responsible for the metabolism of parabens and glucuronides and sulphonate esters are formed subsequently, via involvement of various enzymes (based on data from dermal exposure, summary in SCCS/1446/11).

2.2. Oestrogenic activity

It has been demonstrated that parabens bind to oestrogen receptors with an affinity that is 10,000-, 30,000-, 150,000- and 2,500,000-fold weaker (for butyl, propyl, ethyl and methylparaben, respectively) than that of the natural ligand, 17β-oestradiol (Routledge et al, 1998). Hence, the binding affinity measured increased with chain length (methyl < ethyl < propyl < butylparaben). This study showed also that the metabolite PHBA had no affinity for the oestrogen receptors. Another in vitro study showed that parabens display similar affinity for the 2 types of human oestrogen receptors (ERα and ERβ) (Okubo et al, 2001, Blair et al, 2000). Whereas these receptor binding tests do not enable to differentiate agonist and antagonist activities, further studies tested the transactivating potency of parabens and showed that methyl-, ethyl-, propyl- and butylparaben exerted an agonistic effect on the oestrogenic receptor (Routledge et al, 1998). However, the effect occurred at paraben concentrations 10,000- to 1,000,000-fold greater than those of oestradiol. Yeast cells transfected with the human oestrogen receptor alpha, butyl-, propyl-, ethyl- and methylparaben showed relative responses which were 4000, 8000, 30,000, 200,000 and 3,000,000-fold weaker than 17β-oestradiol, respectively (Miller et al, 2001). Similarly, parabens stimulated the proliferation of a breast tumour cell line over-expressing oestrogen receptors (MCF-7) but the potency was 10,000- to 10,000,000-fold lower than for 17β-oestradiol (Okubo et al, 2001).

In order to test the ability of parabens to induce an oestrogen-type response in an organ sensitive to oestrogen stimulation, uterotrophic assays were performed in immature or ovariectomized female rodents (reviewed by Boberg 2010). Ethylparaben, propylparaben, isopropylparaben, butylparaben and isobutylparaben gave rise to oestrogenic activity (increased uterus weight) whereas methylparaben induced variable responses. The metabolite PHBA was devoid of effects following subcutaneous administration. In the immature mouse, PHBA had an oestrogenic effect, in contrast to what was observed in the rat. Methylparaben and butylparaben induced variable responses in immature mice whereas ethylparaben, propylparaben, butylparaben and isobutylparaben increased uterus weight. The oestrogenic activity occurred after administration of paraben doses 1,000- to 6,000-fold greater than the oestradiol-17β dose.

Another finding indicating that parabens possess some oestrogenic activity is that parabens have been shown to inhibit human cytosolic sulfotransferases (SULTs). SULTs are involved in the sulfonation of 17-β oestradiol causing oestradiol inactivation hence inhibition of sulfonation induces an increase in the quantity of active oestradiol (Harris et al, 2005). The results showed that the potency of SULT inhibition increased as the paraben carbon chain length increased. Butylparaben was found to be the most potent of the parabens in skin cytosol, yielding an IC50 value of 37 µM (Prusakiewicz et al, 2007).

The metabolite PHBA is a common metabolite for all parabens and therefore cannot be accountable for any significant endocrine disrupting effect given the large differences in effects between parabens of increasing ester chain length in various in vitro and in vivo models (SCCS/1446/11). Downstream metabolites consist of various glucuronides and sulfate esters, and also those are not considered likely to have any endocrine disrupting effects.

To conclude, parabens have been associated with oestrogenic activity in in vitro and in vivo models with the potency increasing with paraben carbon chain length. Still, the reported oestrogenic activities were considerably lower (1000 – 10,000,000-fold) than observed for the reference compound 17-β oestradiol.

2.3. Developmental toxicity in males

Methylparaben

No effect on reproductive organ weight, spermatozoid count, or plasma luteinising hormone (LH), follicle stimulating hormone (FSH) or testosterone concentrations was observed when juvenile male rats were exposed via the diet for 8 weeks to methylparaben at approximate dose levels of 10 and 1000 mg/kg/day (Oishi, 2004). Recently, a Good Laboratory Practice (GLP) compliant study, confirmed that methylparaben had no effect on male reproductive organs in the rat (Hoberman et al, 2008).

Propylparaben

A 4-week repeat-dose study conducted on 21 days old juvenile Wistar rats exposed at doses of 0.01, 0.1 or 1% propylparaben in the diet showed an effect on spermatogenesis. A decrease in the testicular and epididymal quantity of spermatozoids was observed with a lowest-observed adverse effect level (LOAEL) of 0.01% corresponding to an average propylparaben intake of 12.4$\pm$3 mg/kg/day. A dose-dependent decrease in serum testosterone concentration was significant at a dose of 1%, corresponding to 125$\pm$30 mg/kg/day propylparaben (Oishi, 2002b).

Recently, another GLP compliant study has been undertaken with a similar but more extensive design. Propylparaben was given by oral gavage to 4 main groups of 20 male Wistar rats at nominal doses of 3, 10, 100 or 1000 mg/kg/day for 8 weeks starting from post natal day (PND) 21. A control group of 20 males received the vehicle. One sub-group of 10 animals per group was necropsied at the end of the dosing period and the other after a 26-week treatment-free period. The following endpoints were assessed: morbidity/mortality, clinical condition, body weight, sexual maturation, LH, FSH and testosterone levels, organ weights, gross and microscopic pathology and sperm quality. Blood samples were taken from additional satellite animals at specific time-points after dosing on PND 21 and PND 77 for toxicokinetics. There were no unscheduled deaths and no remarkable clinical changes in any group throughout the study. Similarly, there were no compound-related organ weight, macroscopic or microscopic changes in the testes and epididymides, and no evidence of an effect on sexual maturation, hormone levels, sperm count or motility, in any group at the end of the treatment and treatment-free periods. The toxicokinetic data showed that the duration of exposure between dosing

intervals was short; non-conjugated propylparaben was detected up to at the most 1 h (after 8 weeks dosing) - 4 h (data after first dose) after dosing in the highest dose group. If total concentrations (non-conjugated and a sulphoconjugate of propylparaben) are considered, exposure was evident for up to 4 h (after 8 weeks dosing) - 8 h (data after first dose). The nominal dose of 1000 mg/kg/day was the no observed effect level (Gazin V. et al, submitted for publication).

2.4. Developmental toxicity in females

In a recent non-GLP study, the potential for parabens to affect reproductive parameters in female juvenile rats was evaluated (Vo et al, 2010). Female rats were treated orally (gavage) with 62.5, 250 and 1000 mg/kg of either methyl-, ethyl-, propyl-, isopropyl-, butyl-, or isobutylparaben from PND 21 to 40. Vo and co-workers demonstrated in vitro that the relative binding affinity to the ERα and ERβ receptors increased with increasing paraben carbon chain length and branching, although the paraben showing the highest affinity (isobutyl), was at least 500 fold less potent than ethinylestradiol. However, this finding was not clearly translated to oestradiol-like effects in the in vivo setting for most parameters studied. The exception was effects on myometrial thickness and on the number of corpora lutea, where parabens with longer ester chain induced some changes in a dose related manner. No effect was observed with methylparaben. For propylparaben, increased myometrial hypertrophy was observed at 1000 mg/kg, while no significant effect was observed on the number of corpora lutea. In conclusion, no consistent effects were observed with methylparaben whereas propylparaben seemed to induce myometrial hypertrophy without any effect on uterus weight with a NOEL of 250 mg/kg.

Studies of the embryo-foetal development with methylparaben in the rat, mouse, hamster and rabbit indicated no effects up to the highest dose levels tested, i.e., 300 (rabbit) and 500 (rodent) mg/kg/day (EFSA review, 2004). Similar studies with propylparaben are lacking.

3. Risk assessment

Background

Risk assessments on parabens have been performed by several European expert panels including the European Food Safety Authority (EFSA) and the Scientific Committee on Consumer Safety (SCCS). EFSA established a full-group acceptable daily intake (ADI) of 0-10 mg/kg body weight for the sum of methylparaben, ethylparaben and propylparaben. The EFSA opinion dated July 2004 considered that propylparaben should not be included anymore in this group ADI due to effects on the male reproductive organs observed in juvenile rats and the lack of a clear NOAEL. As a consequence, from year 2006, propylparaben was no longer allowed for use as a food additive within the European Union (Directive 2006/52/EC).

Parabens are currently authorised in cosmetics at a maximum use concentration of 0.4% (acid) for a single and 0.8% for a mixture of parabens, respectively (directive 76/768/EEC). The SCCS opinion dated March 2011 proposes to modify these levels, considering the use of propylparaben and butylparaben in cosmetics as safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19%. This value is derived from a rat neonatal study where no effects were observed on testis (Fisher et al, 1999).

Methylparaben

Based on *in vitro* data, methylparaben does not display a significant activity at the oestrogenic receptors. Moreover, methylparaben has not been associated with adverse effects on the male reproductive organs in juvenile rats (applying doses up to 1 g/kg) or in embryo-foetal development studies conducted in rodents and non-rodents. Both studies in juvenile rats (Oishi, 2004 and Hoberman

et al, 2008) have been criticized for various reasons (see SCCS/1348/10), including the fact that safety margins cannot be calculated due to the lack of plasma exposure data. However, based on the totality of the *in vitro* and in vivo data, it can be concluded that methylparaben seems to be devoid of adverse effects on reproduction and development.

EFSA has established a full-group ADI of 0-10 mg/kg body weight for the sum of methyl and ethyl parabens and their sodium salts (Directive 2006/52/EC). This limit is considered applicable also for medicinal products and precludes the need for another (PDE) calculation based on ICH Q3C. The use of methylparaben of up to 0.2% as excipient in medicinal products is consistent with this limit.

Propylparaben

Reduced spermatogenesis and serum testosterone level were observed following 4 weeks dietary dosing with 0.01, 0.1 and 1% propylparaben in the diet to PND 21 rats (Oishi, 2002b). The lowest-observed adverse effect level (LOAEL) detected in this study was 0.01% corresponding to 12.4 mg/kg/day.

Such effects were not reproduced in a recent GLP-compliant study (Gazin et al), the design of which is more extensive. Hence, no effects on male reproduction parameters were seen following 8 weeks daily oral administration of doses up to 1000 mg/kg, to male rats from 3-11 weeks of age.

Different oral administration methods were applied in the Oishi (2002b) study and the recently conducted study; via the diet and gavage, respectively. From the Oishi (2002b) study, there are no data on the systemic exposure of the animals, which is a major limitation. Toxicokinetic data from the recently conducted study showed that the duration of exposure between dosing intervals was short. There are no adequate human data on the pharmacokinetic profile of orally administered propylparaben, e.g., following intake of a propylparaben containing pharmaceutical. However, based on data available, it can be anticipated that the systemic exposure to propylparaben following oral intake, at least in adults, is short. In addition, the metabolites are not considered likely to have endocrine disrupting properties, and consequently, the exposure to propylparaben is the main focus. While it is likely that dietary administration of propylparaben, as in the Oishi study, could have resulted in a more prolonged and even systemic exposure to propylparaben, gavage administration, as in the recently conducted study, more closely mimics the clinical setting following oral administration of a medicinal product. In addition due to the design and GLP conditions of the Gazin et al study, its results are considered to be more reliable. Thus, for oral administration of those pharmaceuticals which result in short (hour) daily (but repeated) exposure to propylparaben, the data from the newly conducted juvenile toxicity study provide reassurance regarding lack of risk for endocrine disrupting effects for propylparaben.

Regarding effects on female reproductive system development, a juvenile study using female rat showed limited effects of parabens. Propylparaben seemed to induce myometral hypertrophy at 1000 mg/kg with a NOEL of 250 mg/kg.

Administration of propylparaben containing medicine to children

In the propylparaben study described by Gazin et al dosing of rats started on PND 21 and lasted for 8 weeks. This treatment period corresponds to a human developmental period from approximately 2 years up towards early adulthood, and includes critical development steps i.e. postweaning androgen secretion, prepubertal testosterone surges, development of secretory activity of seminal vesicles, decline in FSH responsiveness, replication of Leydig cells and initiation of spermatogenesis (Klonisch et al, 2004; Marty et al, 2003). This prepubertal period is considered as a sensitive period for studying potential effects on the male reproductive system development (Cortes et al, 1987; Müller et al, 1992). Although not addressing the neonatal period, there is support from the scientific literature that the

male reproductive system is not more sensitive in children below 2 years of age. Thus, in terms of the exposure during critical periods of development, the data from this study can be considered relevant also for children below 2 years of age at equivalent exposures.

However, a more extended exposure to propylparaben cannot be excluded after daily oral administration in very young children such as neonates, since they are anticipated to have less developed metabolic capacity of key enzymes involved in the metabolism of propylparaben, compared to adults. For these youngest children, adequate information regarding exposure to propylparaben after oral intake of a medicinal product containing propylparaben appears to be lacking. Thus, it is not possible to conclude that the data from the new study are fully reassuring for this low age group. Regarding estimation of an acceptable amount of propylpraben that can be included in an oral medicinal product, margins cannot be estimated based on toxicokinetics, given the lack of adequate human data.

On basis of a NOEL for propylparaben of 250 mg/kg/d derived in the Vo et al study (2010), a permitted daily exposure (PDE) for adults and metabolically mature children can be calculated according to the method outlined in ICH Q3C[1]. The following uncertainty factors are used: F1=5 (rat), F2=10 (interindividual variation), F3=1 (exposure that covers juvenile period), F4=1 (lack of severity) and F5=1 (NOAEL available). This calculation gives rise to a PDE for propylparaben in adults and metabolically mature children of 5 mg/kg/d (250 mg/kg/d/ 5x10).

4. Conclusion

General considerations

The CHMP article 5(3) scientific opinion on 'The Potential Risks of Carcinogens, Mutagens and Substances Toxic to Reproduction When These Are Used as Excipients in Medicinal Products for Human Use' states under section 4. "Any risk identified for an excipient and in particular a CMR substance, would be acceptable only on condition that this excipient cannot be substituted with a safer available alternative, or that the toxicological effects in animal models are considered not relevant for humans (e.g. species specific, very large safety ratio) or where the overall benefit/risk balance for the product outweighs the safety concern with the product".

As an antimicrobial preservative, the EMA Guideline on Excipients in the dossier for application for Marketing Authorisation of a Medicinal Product (EMEA/CHMP/QWP/396951/2006) states in its introduction: "Antimicrobial preservatives are normally added to prevent microbial proliferation arising under in use conditions. These properties are due to certain chemical groups which are usually harmful to living cells and might therefore be associated with certain risks when used in humans. Thus inclusion of antimicrobial preservatives or antioxidants in a medicinal product needs special justification. Wherever possible the use of these substances should be avoided, particularly in case of paediatric formulations. The concentration used should be at the lowest feasible level."

Specific considerations

In oral pharmaceutical formulations, combinations of methylparaben and propylparaben are applied with concentrations generally ranging from 0.015 to 0.2% for methylparaben and 0.02% to 0.06% for propylparaben. Based on the current posology of medicines containing propylparaben, a concentration of 0.06% would correspond to a maximal oral intake of propylparaben of approximately 50 mg/day (or 1 mg/kg/day when based on a patient weighing 50 kg).

[1] ICH Topic Q3C Guideline for Residual Solvents defining the Permitted Daily Exposure PDE as the maximal dose level without any toxicity in the animal divided by safety/uncertainty factors: PDE = NOAEL / F1 x F2 x F3 x F4 x F5 with F1 = inter-species extrapolation, F2 for inter-individual variability, F3 for adequacy of exposure period/duration of animal study, F4 for severity of the toxicity, An additional factor F5 = 10 is applied when only LOAEL has been determined.

Methylparaben has not been associated with adverse effects on the male and female reproductive organs in juvenile rats or in embryo-foetal development studies. This allows concluding that the use of methylparaben in oral formulations up to 0.2% of the product (as within the recommended effective concentrations as a preservative) is not a concern for humans including the paediatric population whatever the age group.

Regarding propylparaben, certain oestrogenic activity has been seen in various experimental settings, but with approximately more than 10,000 fold lower activity than oestradiol in *in vitro* pharmacological models. The *in vivo* effects on sperm counts described in the study by Oishi in a juvenile rat model was not replicated in a more recent, well conducted toxicological study in juvenile rats of the same age as in the Oishi study. This study showed an absence of toxicological effects on the maturation of the male reproductive system, up to the highest dose of 1000 mg/kg/d of propylparaben, thus not indicating any endocrine disrupting potential. Based on the published results on the female reproductive system, a conservative NOEL of 25O mg/kg has been determined for propylparaben.

For children below 2 years a PDE for propylparaben cannot be determined because of uncertainty related to the maturation of the enzymes that metabolize propylparaben as well as the limitation of the available animal data corresponding to the youngest children. However safety margins identified in adults and children older than 2 years are currently reassuring. Nevertheless, for children below 2 years further exposure data for propylparaben are needed. The use of a propylparaben containing formulation for the very young could be justified on a case-by-case basis from a benefit/risk perspective, weighting the need for treatment against the potential risk. This assessment should take into account several factors such as the posology and concentration of propylparaben, the treatment duration, the severity of the disease and availability of alternative treatments.

A PDE value of 5 mg/kg/day can be calculated for the use of propylparaben in adults and children older than 2 years with mature metabolic capacity.

5. References

Aubert N, Ameller T and Legrand JJ (2012). Systemic exposure to parabens: pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration Food Chem Toxicol. 50(3-4): 445-54,

Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ and Pope GS (2004). Concentrations of parabens in human breast tumours. J Appl Toxicol. 24(1):5-13

Boberg J, Taxvig C, Christiansen S and Hass U (2010). Possible endocrine disrupting effects of parabens and their metabolites. Reprod Toxicol. 30(2): 301-12

Charnock C and Finsrud T (2007). Combining esters of para-hydroxy benzoic acid (parabens) to achieve increased antimicrobial activity J Clin Pharm Ther 32(6): 567-72

CHMP Committee for Human Medicinal Products (2007). Article 5(3) scientific opinion (2007) on 'The Potential Risks of Carcinogens, Mutagens and Substances Toxic to Reproduction When These Are Used as Excipients in Medicinal Products for Human Use (EMEA/CHMP/SWP/146166/2007)

Cortes D, Müller J and Skakkebaek NE (1987). Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. Int J Androl 10(4): 589-96.

EFSA European Food Safety Agency (2004). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to para hydroxybenzoates (E 214-219). The EFSA Journal. 83:1-26

EMA European Medicines Agency (2007) Guideline on Excipients in the dossier for application for Marketing Authorisation of a Medicinal Product (EMEA/CHMP/QWP/396951/2006)

Fisher JS, Turner KJ, Brown D and Sharpe RM (1999). Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. Environmental health perspectives. 107(5):397-405

Gazin V., Marsden E., Briffaux J-P (2012), Propylparaben: 8-week postweaning juvenile toxicity study with 26-week treatment free period in male Wistar rat by the oral route (gavage) Poster SOT Annual Meeting San Francisco USA - Abstract ID 2359*327

Guideline on Excipients in the dossier for application for Marketing Authorisation of a Medicinal Product (EMEA/CHMP/QWP/396951/2006).Hoberman AM, Schreur DK, Leazer T, Daston GP, Carthew P, Re T Lorets L and Mann P (2008). Lack of effect of butylparaben and methylparaben on the reproductive system in male rats. Birth defects research. 83(2):123-33

Jones PS, Thigpen D, Morrison JL and Richardson AP (1956). p-Hydroxybenzoic acid esters as preservatives. IIE. The physiological disposition of p-hydroxybenzoic acid and its esters. J. Am. Pharm. Assoc. Sci. Ed. 45:265—273.

Kang KS, Che JH, Ryu DY, Kim TW, Li GX. and Lee YS (2002). Decreased sperm number and motile activity on the F1 offspring maternally exposed to butyl p-hydroxybenzoic acid (butyl paraben). J Vet Med Sci 64, 227-35.

Kiwada H. Awazu S and Hanano M. (1980) The study on the biological fate of paraben at the dose of practical usage in rat. II. The pharmacokinetic study on the blood concentration after the administration of ethyl paraben or p-hydroxybenzoic acid. J. Pharmacobiodyn. 3(7):353-363.

Klonisch T, Fowler PA and Hombach-Klonisch S (2004). Molecular and genetic regulation of testis descent and external genitalia development. Dev Biol. 270(1): 1-18.

Marty MS, Chapin RE, Parks LG and Thorsrud BA (2003). Development and maturation of the male reproductive system. Birth Defects Res B Dev Reprod Toxicol 68(2): 125-36.

Miller D, Brian B, Wheals BB, Beresford N, Sumpter JP. (2001). Estrogenic activity of phenolic additives determined by an in vitro yeast bioassay. Environ Health Perspect 109:133–138.

Müller J and Skakkebaek NE (1992). The prenatal and postnatal development of the testis. Baillieres Clin Endcrinol Metab 6(2): 251-71.

Oishi S (2001). Effects of butylparaben on the male reproductive system in rats. Toxicology and industrial health. 17(1):31-9

Oishi S (2002a). Effects of butyl paraben on the male reproductive system in mice. Archives of toxicology. 76(7):423-9

Oishi S (2002b). Effects of propyl paraben on the male reproductive system. Food Chem Toxicol. 40(12):1807-13

Oishi S (2004). Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats. Food Chem Toxicol. 42(11):1845-9

Routledge EJ, Parker J, Odum J, Ashby J, and Sumpter JP. (1998). Some alkyl hydroxyl benzoate preservatives (parabens) are estrogenic. Toxicol Appl Pharmacol 153:12–19.

SCCS Scientific Committee on Consumer Safety – European Commission (2011). Clarification on opinion SCCS/1348/10 in the light of the Danisk clause of safeguard banning the use of parabens in cosmetic products intended for children under the three years of age. SCCS/1446/11.

Rowe RC, Sheskey PJ and Owen SC. Handbook of pharmaceutical excipients. Pharmaceutical Press and American Pharmacist Association. Fifth edition, p vi-918, 2006.

Tsukamoto H and Terada S (1964). Metabolism of drugs. XLVII. Metabolic fate of p-hydroxybenzoic acid and its derivatives in rabbit. Chem. Pharm. Bull. (Tokyo) 12:765-769

Vo TTB, et al. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model (2010).Reprod Toxicol, 29(3):306-16