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

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Table of contents

1. Introduction

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, 2012). Based on the current posology of medicines containing methyl- and propylparaben, concentrations of 0.2% and 0.06% would correspond to maximal intakes of approximately 140 mg/day and 50 mg/day, respectively. 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 “Guideline on excipients in the label and package leaflet of medicinal products for human use (CPMP/463/00 Rev.1)” is under revision and a concept paper on the need for such revision has been published in 2012 (EMA/CHMP/SWP/888239/2011). 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 allergenic 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).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.

2. Discussion

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

Animal data

In a recent study (Aubert et al, 2012), the pharmacokinetics 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. Various types of esterases, notably carboxylesterases hCE1 and hCE2, are responsible for the metabolism of parabens. Glucuronides and sulphonate esters are formed subsequently, via involvement of various enzymes (based on data from dermal exposure, summary in SCCS/1446/11). 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.

Recently, a multi-centre, non-interventional, observational study was performed in neonates. Blood samples were taken at the same time as routine samples following administration of medicines containing methyl- and/or propylparaben, and the concentration of each excipient was then determined. For propylparaben (PPB), blood concentrations above the limit of detection (LOD=10 ng/ml) were only observed in 49% (87 out of 181) of patients and only 25 % of all (841) PPB blood concentrations were above the LOD.. Only four samples (from four different patients) were above 100 ng/ml, with the maximal obtained value 147 ng/ml (Mulla et al, 2015).

2.2. Oestrogenic activity

It has been demonstrated that parabens bind to oestrogen receptors with binding affinities increasing with side chain length and branching. In rat oestrogen receptors, Blair et al (2000) measured mean IC_{50} values ranging from $2.45.10^{-4}$ M (methylparaben) to $1.05.10^{-4}$ M (butylparaben) whereas that of the natural ligand 17β-oestradiol was at least 110,000-fold lower (IC_{50} of $8.99.10^{-10}$ M). The same trend was shown by Okubo et al (2001) and Vo et al (2010) with human oestrogen receptors α and β. These authors also demonstrated that parabens display similar affinity for the 2 types of human oestrogen receptors.

Whereas these receptor binding tests do not enable to differentiate agonist and antagonist activities, further studies investigated the transactivating potency of parabens. They demonstrated the ability of methyl-, ethyl-, propyl- and butylparaben to exert an agonistic effect on the oestrogenic receptor which increased concurrently with alkyl group size. However, the potency of these parabens was much lower than that of 17β-oestradiol. In yeast cells transfected with the human oestrogen receptor α, Miller et al (2001) measured EC_{50} values 8000- to 3,000,000-fold lower than that of 17β-oestradiol (2.10^{-10} M), in line with results obtained by Routledge et al (1998). Similarly, Okubo et al. (2001) showed that these compounds are able to stimulate the proliferation of a breast tumour cell line over-expressing oestrogen receptors (MCF-7). Nevertheless, based on the concentration yielding maximal proliferation of cells (C_{max}), their potency was at least 600,000-fold lower than for 17β-oestradiol (C_{max} of 3.10^{-11} M). Watanabe et al (2013) investigated the structure-activity relationship of 17 parabens for estrogenic activity in a luciferase reporter gene assay in CHO-K1 cells transfected with oestrogen receptors α and β. Among the 12 parabens with linear alkyl chains ranging in length from C_1 to C_{12}, the most potent oestrogen receptor agonist activity was measured for heptyl- (C_7) and pentylparaben (C_5). Estrogenic activity decreased in a stepwise manner as the alkyl chain was shortened to C_1 or lengthened to C_{12}.

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). In oral studies performed in immature rodents, methyl-, ethyl-, propyl-, and butylparaben did not produce an increase in uterine weights at doses up to 800, 1000, 100, and 1200 mg/kg, respectively (Hossaini et al, 2000; Routledge et al, 1998). In studies performed by subcutaneous administration, both negative and positive results were reported according to the species and experimental model used, as detailed thereafter. In rats, ethylparaben, propylparaben, butylparaben and isobutylparaben gave rise to oestrogenic activity (increased uterus weight) whereas methylparaben induced variable responses. In immature mice, variable responses were observed for these compounds. In ovariectomized mice, methylparaben, ethylparaben and propylparaben increased uterus weight whereas butylparaben induced variable responses (Hossaini et al, 2000; Lemini et al, 2003; Lemini et al, 2004; Routledge et al, 1998; Shaw et al, 2009). The ED_{50} values determined by Lemini et al (2003) for the uterotrophic effects of parabens in immature mice and rats, and ovariectomized mice were 2,000- to 30,000-fold higher than those determined for 17β-estradiol (6.9 to 17.4 µg/kg) in the same experimental conditions.

Table 1: ER binding and estrogenic activity of methyl- and propylparaben

Experimental model	Endpoint	Variable	Paraben		Reference compound	Source
			Methyl	Propyl		
Affinity for estrogenic receptor						
ER, rat	Binding	IC_{50} (M)	$2.45.10^{-4}$	$1.50.10^{-4}$	E_2:$8.99.10^{-10}$ DES: $2.25.10^{-10}$	Blair et al, 2000
ERα, human	Binding	IC_{50} (M)	>$2.7.10^{-4}$[a]	$9.0.10^{-5}$	DES: 3.10^{-8}	Okubo et al, 2001
ERβ, human	Binding	IC_{50} (M)	>$2.4.10^{-4}$[a]	$6.0.10^{-5}$	DES: $2.6.10^{-8}$	Okubo et al, 2001
ERα, human	Binding	IC_{50} (M)	>$4.63.10^{-5}$[a]	$1.87.10^{-5}$	E_2: $2.99.10^{-9}$	Vo et al, 2010
ERβ, human	Binding	IC_{50} (M)	>$1.65.10^{-5}$[a]	$1.65.10^{-5}$	E_2: $3.03.10^{-9}$	Vo et al, 2010
Estrogenic activity *in vitro*						
Yeast cells transf. with human ERα	β-galactosidase activity	RP[b]	$3.3.10^{-7}$	$3.3.10^{-5}$		Miller et al, 2001
Yeast cells transf. with human ER	β-galactosidase activity	RP[b]	4.10^{-7}	$3.3.10^{-5}$		Routledge et al, 1998
MCF-7 cells	Proliferation	EC_{50} (M)	$3.1.10^{-5}$	$1.9.10^{-6}$	E_2: $1.60.10^{-12}$	Okubo et al, 2001
CHO-K1 cells transf. with ERα	Luciferase activity	REC_{20} (M)[c]	–[d]	$6.1.10^{-7}$	E_2: $2.5.10^{-12}$	Watanabe et al, 2013
CHO-K1 cells transf. with ERβ	Luciferase activity	REC_{20} (M)[c]	–	$1.7.10^{-7}$	E_2: $5.3.10^{-12}$	Watanabe et al, 2013
Estrogenic activity *in vivo* – uterotrophic assays						
Immature mouse	Uterine weight	ED_{50} (mg/kg)	18	17	E_2: 0.0071	Lemini et al, 2003
OVX mouse	Uterine weight	ED_{50} (mg/kg)	35	43	E_2: 0.0069	Lemini et al, 2003
Immature rat	Uterine weight (dry)	ED_{50} (mg/kg)	64	33	E_2: 0.0103	Lemini et al, 2003
Immature rat	Uterine weight (wet)	ED_{50} (mg/kg)	79	68	E_2: 0.0174	Lemini et al, 2003

NT: not tested; OVX: ovariectomized
[a] IC_{50} could not be calculated since the binding affinity of methylparaben was too low, and found to be lower than that of ethylparaben (value reported is >IC_{50} determined for ethylparaben)
[b] Relative potency vs. E_2
[c] 20% relative effective concentration (concentration of test compound showing agonistic activity equivalent to 20% of that of 10^{-9}M E_2)
[d] no effect (REC_{20}>10^{-5} M)

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 PHBA metabolite was inactive in the recombinant yeast oestrogen assay (Routledge et al, 1998). Variable results were observed in uterotrophic assays performed in immature mice, whereas no estrogenic activity was reported in immature rats (Lemini et al 1997, Hossaini et al, 2000, Lemini et al 2003). 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 are considerably lower than observed for the reference compound 17-β oestradiol (see above Table 1).

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±3 mg/kg/day. A dose-dependent decrease in serum testosterone concentration was significant at a dose of 1%, corresponding to 1290±283 mg/kg/day propylparaben (Oishi, 2002b).

To confirm these conclusions, a GLP-compliant juvenile toxicity study was performed with a similar but more extensive design. Propylparaben was given by oral gavage to male Wistar rats (20 per group) at doses of 0 (vehicle), 3, 10, 100 or 1000 mg/kg/day for 8 weeks starting from post-natal day (PND) 21. Half of the animals was necropsied at the end of the dosing period, and the other half was necropsied after a 26-week treatment-free period. There were no compound-related effect on organ weight, macroscopic or microscopic changes neither in the testes nor in 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). In conclusion, although exposure to propylparaben was observed following gavage administration to rats, there was no evidence of any effect on male rat reproductive organs. This toxicity study conducted according to GLP in an appropriate and statistically robust manner failed to confirm the effects on endocrine functions observed by Oishi (2002b). The dose of 1000 mg/kg/day was the no observed effect level (Gazin et al, 2013).

An additional GLP-compliant study was conducted to determine the potential toxicity and reproductive effects of propylparaben in juvenile Sprague-Dawley rats (25 per sex per group) treated by oral gavage from PND 4 through 90 at 0 (vehicle), 10, 100, and 1000 mg/kg/day. Male animals were necropsied either at the end of treatment (10 per group), or at the end of a 40-day treatment-free period (15 per sex per group) used to evaluate their reproductive capability when mated with naïve females. In males, there were no compound-related effect on organ weights, no gross or histopathologic findings in reproductive tissues, no effect on sexual maturation, and no evidence of an effect on the reproductive capability (mating and fertility indices, number of days to mating, conception rate). Toxicokinetic investigations were conducted on PND 7, 21, and 83. Exposure levels to propylparaben and three of its metabolites (p-hydroxybenzoic acid, sulfate of propylparaben, sulfate of p-hydroxybenzoic acid) were greater in 7-day old rats and decreased with increasing age. The duration of exposure between dosing intervals was inversely correlated to the age, in line with ongoing metabolic maturation from 7 days to 83 days of age. In the highest dose group, non-conjugated propylparaben was detected up to 24h, 8h, and 8h after dosing on PND7, 21, and 83, respectively. At

the same time, metabolites were detected up to 24h, 24h and 8 hours, respectively after dosing on PND7, 21, and 83, respectively. In all dose groups and age subsets, propylparaben was a minor circulating analyte with mean systemic exposures that were less than 1% of total measured AUC. Overall, there were no propylparaben-related changes on a range of endpoints that could be suggestive of an estrogenic effect, and the dose of 1000 mg/kg/day was the no observed effect level in males (Pouliot, 2013). This study confirms the results obtained by Gazin et al (2013) and even extend them since rats were dosed from the neonatal period. It also strengthens the NOEL value of 1000 mg/kg/day of propylparaben for juvenile male rats.

2.4. Developmental toxicity in females

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.

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 affinity of 17β-estradiol was at least 500-fold higher than that of the paraben showing the highest affinity (isobutyl). 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 at up to 1000 mg/kg/day whereas propylparaben seemed to induce myometrial hypertrophy at this high dose level.

As mentioned in section 2.3. Pouliot (2013) performed a GLP-compliant juvenile toxicity study in juvenile Sprague-Dawley rats treated with propylparaben by oral gavage from PND 4 through PND 90 at 0 (vehicle), 10, 100, and 1000 mg/kg/day (25 rats per sex per group). Female animals were necropsied either at the end of treatment (10 per group), or at the end of a treatment-free period (15 per sex per group) used to evaluate any effect on their reproductive capability when mated with untreated proven breeder males. In the latter case, females and their pups were sacrificed 5 to 7 days post-partum. Most parameters investigated remained unaffected by treatment with propylparaben at any dose level, notably: histology of reproductive tissues, oestrous cyclicity, mating and fertility, maternal performance, and parameters evaluated on pups (birth weight, litter size, viability, clinical observations, external malformations). However, significant effects were observed on the onset of puberty (accelerated) and on the weight of uterus (increased) at 1000 mg/kg/day. Toxicokinetic parameters measured in females were not significantly different from those measured in males, therefore results reported above (see section 2.3. The exception was that 21-day old females were exposed to non-conjugated propylparaben for a longer duration after dosing on PND 21 (detected up to 24h after dosing at 1000 mg/kg/d). Overall, the dose of 100 mg/kg/day was the no observed effect level in females since propylparaben-related changes suggestive of an estrogenic effect were observed at 1000 mg/kg/day.

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).

Methylparaben and ethylparaben are currently authorised in ready for use cosmetic preparations at a maximum use concentration of 0.4% (as acid) for a single and 0.8% (as acid) for a mixture of parabens, respectively. The use of propylparaben and butylparaben in such cosmetic products is considered as safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.14% (as acid). This value is derived from a rat neonatal study where no effects were observed on testis (Fisher et al, 1999). However, both compounds should not be used in leave-on cosmetic products designed for application on the nappy area of children below three years due to concerns related to metabolic immaturity and possibility of damaged skin in this area (Annex V to Regulation (EC) No 1223/2009).

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 up to 0.2% as excipient in medicinal products is consistent with this limit since it would correspond to a maximal intake of approximately 140 mg/day (2.8 mg/kg/day based on an individual body weight of 50 kg).

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 confirmed in a recent GLP-compliant study (Gazin et al, 2013), the design of which is more extensive. In addition, no effect on the developing male reproductive tract and no effect on male fertility could be observed when male rats were treated from the neonatal period (Pouliot, 2013). Hence, it is considered that propylparaben did not cause any effect on male reproduction parameters following daily oral administration of doses up to 1000 mg/kg to male rats from 4 to 90 days of age.

Regarding effects on female reproductive system development, a non-GLP 3-week juvenile study using female rats showed limited effects of parabens. Propylparaben seemed to induce myometral hypertrophy at 1000 mg/kg/day (Vo et al, 2010). This finding was not confirmed in a recent GLP-compliant 3-month juvenile toxicity study using a more extensive study design, i.e. from the neonatal period up to adulthood (Pouliot, 2013). However, propylparaben-related changes suggestive of an estrogenic effect were observed at the high dose level, *i.e.* earlier onset of puberty and increased weight of uterus, without any concomitant effect on the histology of reproductive tissues, oestrous cyclicity, mating and fertility, and maternal performance. The NOEL was determined at 100 mg/kg/day.

Different oral administration methods were applied in the Oishi (2002b) study, and in the studies conducted by Gazin et al (2013) or Pouliot (2013); via the diet and gavage administration, 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 Gazin et al (2013) showed that the duration of exposure between dosing intervals was short when animals were dosed from weaning, whereas Pouliot (2013) showed prolonged exposure of animals dosed during the neonatal period. Although there are no adequate human data on the pharmacokinetic profile of orally administered propylparaben, e.g., following intake of a propylparaben containing pharmaceutical, the most recent juvenile toxicity studies include gavage administration in order to more closely mimic the oral administration of propylparaben in pharmaceuticals (Gazin et al, 2013; Pouliot, 2013). 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. In addition due to the design and GLP conditions of the two studies performed by oral gavage, their 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 recently conducted juvenile toxicity studies were useful to determine thresholds to assess the risk for endocrine disrupting effects of propylparaben.

Administration of propylparaben-containing medicines to children

In the propylparaben study described by Pouliot (2013), dosing of male and female juvenile rats started on PND 4 and lasted for 3 months. This treatment period corresponds to a human developmental period from birth up towards adulthood. It includes critical developmental steps of male and female reproductive organs, and maturation of pathways involved in the metabolism of parabens. Therefore, the data from this study are considered as relevant for all paediatric subsets.

Regarding estimation of an acceptable amount of propylparaben that can be included in an oral medicinal product, margins cannot be estimated based on animal-to-human exposure comparison due to insufficient human data. Indeed, the number of positive samples in the neonates study published by Mulla et al (2015) was not sufficient to establish a kinetic model in human neonates for propylparaben.. On basis of a NOEL for propylparaben of 100 mg/kg/d derived in the Pouliot study (2013), a permitted daily exposure (PDE) for adults and paediatric patients 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 (NOEL available). This calculation gives rise to a PDE for propylparaben in adults and paediatric patients of 2 mg/kg/d.

[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 = NOEL / 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.

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 methylparaben and propylparaben[2], maximal concentrations of 0.2% and 0.06% would correspond to maximal oral intakes of methylparaben and propylparaben of approximately 140 mg/day and 50 mg/day, respectively(or 2.8 mg/kg/day and 1 mg/kg/day when based on a patient weighing 50 kg).

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 much 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. The lack of effect of propylparaben on the maturation of the male reproductive system at up to 1000 mg/kg/day was confirmed further in another juvenile toxicity study using rats treated from the neonatal period. In female rats, changes suggestive of an estrogenic effect were observed at 1000 mg/kg/day, *i.e.* earlier onset of puberty and increased weight of uterus. There was no concomitant effect on the histology of reproductive tissues, oestrous cyclicity, mating and fertility, and maternal performance of these animals at up to 1000 mg/kg/day.

Based on the results on the female reproductive system, a conservative NOEL of 100 mg/kg/day has been determined for propylparaben. A PDE value of 2 mg/kg/day can be calculated for the use of propylparaben in adults and paediatric patients.

[2] Based on a survey conducted on human oral medicinal products approved in France

Following this review, additional information for parabens in the “Guideline on excipients in the label and package leaflet of medicinal products for human use (CPMP/463/00 Rev.1)” is not considered necessary due to the absence of sufficient clinical evidence of parabens-related effects in humans.

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