ORIGINAL ARTICLE

ESTROGENIC BURDEN OF PARABENS USED IN CHILD CARE PRODUCTS

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Abstract

Parabens are extensively used in personal care products as preservatives but the current regulation of cosmetics (Directive 76/768/EEC) did not take into account their potentially endocrine-disrupting effects. Therefore we performed an assessment of the most used parabens (methyl - and *n*-propylparaben) investigating their estrogenic burden. Systemic exposure dose *via* cosmetics was estimated using two approaches: *ConsExpo* modeling and bio-monitoring data. The endocrine-disrupting effects (uterotrophic assay) were selected as the paraben's critical endpoints and using the assumption of the dose addition for combined effects of parabens, due to their toxicological similarity to estradiol, exposure was calculated in terms of estradiol and compared with the physiological ranges.

Rezumat

Parabenii sunt larg utilizaţi cu rol de conservaţi în produsele de îngrijire personală, dar actuala legislaţie în domeniul produselor cosmetice (Directiva 76/768/EEC) nu are în vedere şi potenţialele lor efecte perturbatoare la nivel endocrin. De aceea am evaluat cei mai utilizaţi parabeni (metil - şi *n*-propilparabenul), investigând efectul lor perturbator endocrin şi încărcarea estrogenică. Doza de expunere sistemică prin produse cosmetice a fost estimată prin două modalităţi: modelare *ConsExpo* şi prin biomonitorizare. Efectele perturbatoare endocrine (testul uterotrofic) au fost selectate ca fiind critice iar, presupunând că în cazul asocierii parabenilor există o aditivitate a efectului datorită similarităţii toxicologice cu estradiolul, expunerea a fost calculată în unităţi de estradiol şi comparată cu nivelele fiziologice ale acestuia.

Keywords: parabens, endocrine disruptors, estrogenic burden

Introduction

Parabens (alkyl esters of *p*-hydroxybenzoic acid) are extensively used in child care products as preservatives, but their safety is not clearly established [2, 6, 11]. Recently, a survey carried out by the Danish Environmental Protection Agency on cosmetic products, showed that parabens are highly used in products topically applied in children [3]. More than 23% of the sunscreens and more than 11% of body lotions contained methylparaben or *n*-propylparaben. The survey also demonstrated the preferential use of these two compounds.

According to annex VI, part I of the European Cosmetic Directive 76/768/EEC, the use of parabens in cosmetic products is allowed with a maximum concentration of 0.4% for each and a total maximum concentration of 0.8% [8].

In December 2010, the Scientific Committee on Consumer Safety (SCCS) published an updated opinion on parabens, concluding that there is no enough data to perform a risk assessment for propyl- and butylparaben in humans [13] and proposed a maximum limit of 0.19% for each paraben.

On the 8th of March 2011, the Council of the European Union noted that Denmark bans the use of *n*-propyl- and butyl-paraben in products used by children under three years old. This decision was, based on their potentially endocrine-disrupting effects.

Starting from this point, we performed a risk assessment of the most used parabens (methyl and *n*-propylparaben) focusing on their endocrine disrupting effects in children and investigated the estrogenic burden.

Materials and Methods

For the exposure in children *via* cosmetics we used two approaches to estimate the systemic exposure dose (SED): *ConsExpo* modeling and extrapolation based on bio-monitoring data.

Estimation of SED using the software model ConsExpo

The software model *ConsExpo (version 4.1),* developed by the Dutch National Institute for Public Health and Environment, contains a set of general models that enables the estimation of exposure to substances from consumer products that are used indoor and their uptake by humans. Estimation by *ConsExpo 4.1* was done for different scenarios that imply different habits, influenced by age and nationality. Scenarios included dermal exposure during summer and winter periods, for a baby of 4.5 months old, a child of 2 or 6.5 years old from the Netherlands or Denmark.

The quantities used are according to *Technical Guidance Document on Risk Assessment* [7]. For the rinse-off products a dilution factor of 0.01 was taken into account, while for stay-on products was used a factor of 1. The absorption coefficient used (0.035, meaning 3.5%) is similar with the one established by SCCS [12]. In all scenarios, the concentrations of methyl- and *n*-propyl parabens in products are set at 0.32% for each, taking into account the real cases measured [12].

Extrapolation based on bio-monitoring data

Estimated internal dose (expressed as µg/kg bw/day) was calculated using the published bio-monitoring data [4]. Urinary excretion levels of total methyl paraben (50^{th} percentile -25 µg/L and 95^{th} percentile -1560 µg/L) were higher than total n-propyl paraben levels (50^{th} percentile 2.5 µg/L and 95^{th} percentile -125 µg/L). Total parabens levels in urine are expressed as free and conjugated parabens and *p*-hydroxybenzoic acid. Extrapolation of bio-monitoring data to internal dose was done taking into account the structural similarity of parabens with diethylphthalate (both have a similar ester functional group and almost the same molecular weight) and their metabolism similarity [5]. The estimation was done in 2 steps: (1) by calculating the ratio of estimated internal dose/exposure to diethylphthalate (0.110 mg/kgbw/day) to the 95^{th} percentile total diethylphthalate urinary level of 3750 ng/mL [5] and (2) by applying this clearance factor to the 95^{th} percentile total methyl- or *n*-propylparabens urinary levels reported in children [4] in order to calculate the internal dose for each parabens.

Results and Discussion

An example of systemic exposure dose (SED) calculated using *ConsExpo 4.1* is presented in Table I. This was done for all scenarios mentioned before and the comparative results are presented in Table II.

Table I

ConsExpo results for the systemic exposure dose (SED) for a child of 2 years old-body surface: 6000 cm^2; body weight: 15.2 kg

Product		**Amount applied (mg)**	**Surface (cm^3)**	**Uses per year**	**Time (min)**	**Dilution factor**	**Absorption coefficient**	**SED (µg/kg bw/day) for each paraben**	
								Denmark	***The Netherlands***
Body lotion	Methylparaben or propylparaben	2700	6000	256	720	1	0.035	8.53	14.1
Shampoo		6000	94	260	4	0.01	0.035	0.19	0.31
Baby cream		270	190	730	720	1	0.035	-	3.98
Liquid soap		2500	295	1825	1	0.01	0.035	-	0.92
Liquid soap shower		13000	6000	329	4	0.01	0.035	0.41	0.86
Sunscreen (full body)		18000 /10000	6000	28	150	1	0.035	10.2	15.1
Sunscreen (face, arms, legs)		9000	2900	28	-	1	0.035	5.08	-
Sunscreen (face,arms)		3600	1200	98	-	1	0.035	7.12	-
Toothpaste		860	300	365	-	-	1	-	63.15
Total summer								**31.53**	**98.42**
Total winter								**9.13**	**83.32**

Table II

ConsExpo results for different scenarios in both countries and at different ages

SED (µg/kg bw/day) of each paraben							
Product	**Metyl paraben or n-propyl paraben**	**4.5 months old**		**2 years old**		**6.5 years old**	
		Denmark	***The Nederlands***	**Denmark**	***The Nederlands***	**Denmark**	***The Nederlands***
Shampoo		0.31	0.51	0.19	0.31	0.14	0.23
Body lotion		-	-	8.53	14.1	-	-
Baby care products		37.83	37.83	-	3.98	-	-
Liquid soap		0.58	1.22	0.41	1.78	0.3	1.4
Sunscreen		12.6	6.42	22.4	15.1	19.59	11.1
Toothpaste		-	-	-	63.15	40.07	40.07
Total winter		**38.72**	**39.56**	**9.13**	**83.32**	**40.51**	**41.7**
Total summer		**51.32**	**45.98**	**31.53**	**98.42**	**60.1**	**52.8**

Comparing the estimated results, for 4.5 months old and 6.5 years old (see Table II), there are no significant differences between children that are living in Denmark or Netherlands (38.72 *vs.* 39.56 µg/kg bw/day for 4.5 months old; 40.51 *vs.* 41.7 µg/kg bw/day for 6.5 years old), but for the age corresponding to 2 years old, the daily uptake is nine times higher in Netherlands (83.32 µg/kg bw/day *vs.* 9.13 µg/kg bw/day), because the scenario implies the use of toothpaste, while in Denmark technical guidance document did not mention the exposure *via* oral route. For the same scenario (in Netherlands) the daily uptake is reduced as the age is increasing, mainly to the fact that the quantity of toothpaste ingested is decreasing. For all ages, exposure is higher in the summer period than in winter, based on the specific use of sunscreen protection products.

Estimated internal doses

The calculated value for clearance factor of diethylphthalate, corresponds to 0.029 mL/kg bw/day. Applying this value to the 95th percentile total methyl- or propyl parabens urinary levels reported in children of 6-11 years old (1560 µg/L and 125 µg/L, respectively), the estimated internal doses correspond to 45.76 µg/kg bw/day for methylparaben and 3.66 µg/kg bw/day for *n*-propylparaben. The following limitation of this extrapolation should be noted: this is aggregate exposure (taking into account all sources and routes of exposure), therefore exposure *via* dermal route is considerably lower.

Comparison of the results (modeling vs. estimated internal doses)

Comparing the *ConsExpo* results obtained in Netherlands during winter for the age of 6.5 years old with the one obtained based on bio-monitoring data on a child of 6-11 years old (see Table III), the conclusion is that *ConsExpo* results are realistic at least for the daily uptake of methylparaben (41.7 µg/kg bw/day *vs.* 45.76 µg/kg bw/day).

Table III

ConsExpo modeling (for 6.5 years old) vs. estimated internal doses

Compound	ConsExpo scenario	Systemic exposure dose (dermal exposure)		Estimated internal dose (aggregate exposure)
		Winter	Summer	
Methyl paraben	Denmark	40.51 µg/kg bw/day	60.1 µg/kg bw/day	**45.76 µg/kg bw/day**
	The Nederlands	41.7 µg/kg bw/day	52.8 µg/kg bw/day	
n-Propyl paraben	Denmark	40.51 µg/kg bw/day	60.1 µg/kg bw/day	**3.66 µg/kg bw/day**
	The Nederlands	41.7 µg/kg bw/day	52.8 µg/kg bw/day	

For *n*-propylparaben, *ConsExpo* results overestimate the real uptake (41.7 µg/kg bw/day *vs.*3.66 µg/kg bw/day from bio-monitoring). This might be caused by the fact that real concentrations of *n*-propylparaben in products are lower than 0.32% or the absorption is lower than 3.5%. This is plausible, considering the lack of *in vivo* human absorption data.

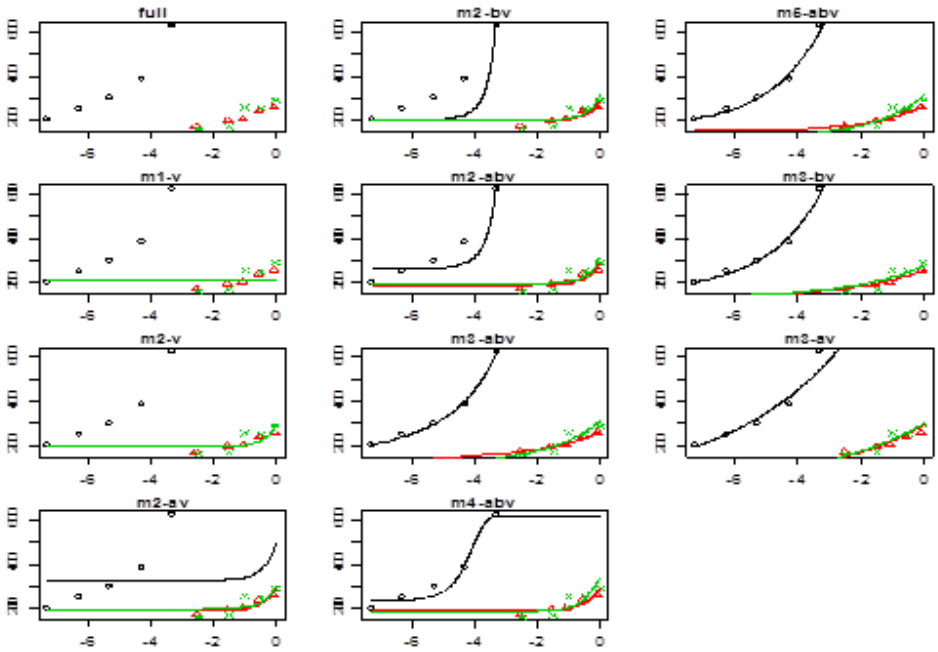

Figure 1.

PROAST modeling (determination of ED_{50} values); black-E2; red: methylparaben; green: propylparaben

Estrogenic burden of parabens

Taking into account that the real exposure is to a mixture of parabens and not to a single compound, we used the toxicological similarity (regarding estrogenic effects) between parabens and following the flow chart approach for mixtures, developed by US EPA [13] we calculated the effect of the mixture as the estrogenic burden and compared the results to physiological levels of oestrogen in the prepubertal child. The estrogenic effect factors (EEF) of parabens were determined using *in vivo* data on uterotrophic assay [9, 10]. EEF were calculated according to the formula: EEF = $ED_{50(estradiol)}/ED_{50(paraben)}$, where ED_{50} represents the dose that induces an increase of uterine weight with 50% [14]. Using PROAST software, *vers.* 28.1, that plotted dose-response curves and the published data [9, 10], we calculated ED_{50} values in immature mice. For the statistical analysis of dose-response data, input data were continuous, summary data (response for uterotrophic assay), while the independent variable was the dose and the covariate was the compound (variable 1-estradiol; variable 2-methyl- paraben; variable 3- propylparaben).

The selected model by the software was m3-abv (Figures 1 and 2), and the ED_{50} values (picked for the lower bound that includes all uncertainties) correspond to 6.93 µg/kg bw 18.10 mg/kg bw and 17.13 mg/kg bw for estradiol, methylparaben and propylparaben, respectively.

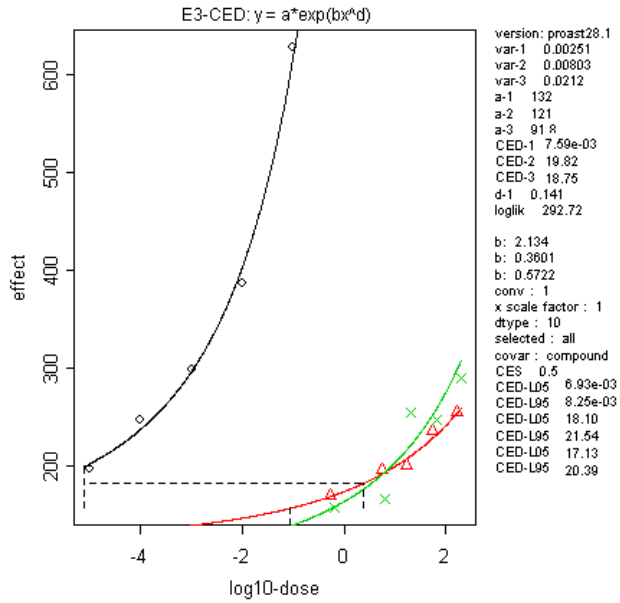

Figure 2.
Selected model by PROAST (determination of ED_{50} values)

Based on these data, calculated EEF corresponds to 3.6 x 10^{-4} for methylparaben and to 3.82 x 10^{-4} for propylparaben. The EEF values are comparable, meaning that methyl- and propylparaben estrogenic potencies *in vivo* are comparable.

Table IV
Estrogenic burden of parabens

	Scenario		**Exposure (µg/kg bw/day) for each paraben**	**Total exposure in terms of E2**	
4.5 months old	The Nederlands	Winter	39.56	0.032 µg/kg bw/day (0.42 pg/mL)	**Physiological levels of E2[1]**: 0.27- 2.72 pg/mL; Mean value: 1.36 pg/mL
		Summer	45.98	0.037 µg/kg bw/day (0.49 pg/mL)	
	Denmark	Winter	38.72	0.031 µg/kg bw/day (0.41 pg/mL)	
		Summer	51.32	0.040 µg/kg bw/day (0.54 pg/mL)	
2 years old	The Nederlands	Winter	83.32	0.0066 µg/kg bw/day (0.88 pg/mL)	
		Summer	98.42	0.0775 µg/kg bw/day (1.04 pg/mL)	
	Denmark	Winter	9.13	0.0072 µg/kg bw/day (0.096 pg/mL)	
		Summer	31.53	0.025 µg/kg bw/day (0.34 pg/mL)	
6.5 years old	The Nederlands	Winter	41.70	0.0333 µg/kg bw/day (0.44 pg/mL)	
		Summer	52.8	0.043 µg/kg bw/day (0.56 pg/mL)	
	Denmark	Winter	40.51	0.032 µg/kg bw/day (0.43 pg/mL)	
		Summer	60.1	0.048 µg/kg bw/day (0.63 pg/mL)	

Then, the *additivity assumption* between parabens was used, in order to estimate a total estrogenic effect. Taking into account the EEF values, exposure was calculated in terms of estradiol (E2). The calculations were done for each paraben by multiplying the systemic exposure dose (μg/kg bw/day) with the EEF value. Results obtained (as μg/kg bw/day) were converted in pg/mL blood. Results (as pg/mL) are presented in Table IV and were compared with physiological estradiol levels in children [1].

In all cases, parabens exposure expressed in terms of estradiol was equivalent to the physiological ranges of estradiol in children.

Using the estimated internal doses based on bio-monitoring data (45.76 μg/kg bw/day for methylparaben and 3.66 μg/kg bw/day for propylparaben) and applying EEF values, the total exposure in terms of estradiol corresponds to 0.25 pg/mL, so almost the minimum endogenous level.

Conclusions

Systemic exposure doses in children *via* cosmetics, estimated using *ConsExpo* software, were between 31.53 and 98.42 μg/kgbw/day during summer and 9.13-83.32 μg/kgbw/day during winter period, for each paraben. Using the additivity assumption between methyl- and propylparabens and their toxicological similarity, exposure was calculated in terms of estradiol. Values obtained (0.096 pg/mL to 1.04 pg/mL) are included in the physiological range of estradiol, indicating that both parabens may be present in blood samples with estrogenic effects comparable to endogenous estrogens and this is a cause of concern, especially for children.

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