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**The parabens and individual wastewater treatment system: What about  
the role of soil in purifying the treated wastewater?**

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Projet ANCREs





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### **Preamble**

Parabens are commonly used as antimicrobial preservatives in household products, cosmetics, pharmaceuticals, and food and beverage processing, and are environmental compounds with oestrogenic activity. Widespread human exposure to parabens has been recently documented, and some parabens have demonstrated adverse effects on male reproduction in animal studies. Human epidemiologic studies are lacking but from recent studies, parabens appear to be a factor of breast cancer development. Concentrations of single compounds in breast tissue are generally lower than needed for assayable oestrogenic responses. In combination, chemicals can give oestrogenic responses at lower concentrations, which suggest that in the breast, low doses of many compounds could sum to give a significant oestrogenic stimulus. On this basis, an interdiction of these products is rising into debate in France.

Due to an increased use of preservatives, more and more parabens end up in surface and ground waters, since they are not completely metabolised in the human body nor completely removed during wastewater treatment. Since health risks related to ingestion of these molecules via tap water are not yet known, most water utilities want to find techniques to remove these substances from the water. One of the options to remove it is by natural removal during soil passage. When water passes through soil or a river bank for instance, organic pollutants are partially removed by several reactions and processes like: chemical reactions, adsorption, biodegradation, die-off and predation and plant uptake.

Since a long time, the soil depurator function is well known. It has been widely used on sewage works since the beginning of wastewater treatment and has been stepwise introduced as a technical element in individual sanitation in 1920 in France. Individual sanitation (IS) which is the application of the ANCRES project must be addressed in area where sewage pipes don't exist or waste water treatment plants can not be built, due to technical-economic conditions. After a decantation / liquefaction step in a septic tank, pre-treated waste water is led to the soil. In most cases, aerobic digestion and soil infiltration occur in the upper layer of the soil and is still widely

used today. The French IS legislation encourages soakaway as the main way to drain off treated waste water while soil infiltration capability is suitable.

Considering the total European population (460 millions of people), about 46 to 92 millions are in concern of on-site treatment and evacuation through the soil and in France this mode of depuration of domestic wastewater is used by 5-6 millions of houses which is about 20% of population of France. Thus, understanding the impact of domestic on-site waste water treatment on the soil functions is of particular interest. Contamination hazards of groundwater are also to be studied, even in the case of treated waste water infiltration. On-site systems are assumed to be low-cost and efficient enough compared with centralized plants in rural and even peri-urban areas. In a matter of health and environment protection, it could be benefit for the European Community to assess national good practices and widely spread them to the other State-members, furthermore in the most recently members (Eastern Europe) where treatment plants are poorly developed.

Thus, our goal is two-fold, for this quasi ubiquitous use of soils:

A. characterise the ecosystem services – infiltration into the soil; retention of elements, molecules or particles within the soil by percolation in soil; bio-chemical degradation; groundwater recharge through the soil;

B. estimates the spatial extension and intensity of its impact on the soils, surface waters and groundwater.

In order to elaborate an integrated indicator, chemical, physical and socio-technical soil properties are measured either by laboratory experiments or by in-situ tests and the data are collected and managed in a database. At the chemical side, at first, classical analysis on soil properties is performed (pH, Total Organic Carbon, Nitrogen, Phosphorus, ionic exchange capacity...) then, enzymatic activity assays (lipase,  $\beta$ -glycosidase, protease, urease,  $\beta$ -glucosaminidase, acid et alkaline phosphatases and arylsulfatase) on soil samples under infiltration is done, and finally the research is completed by analysis of synthetic chemical compounds as parabens in soil and percolated water samples.

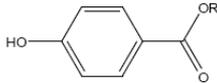
## 1. Introduction

Parabens are commonly used as antimicrobial preservatives in cosmetics, pharmaceuticals, and food and beverage processing. Widespread human exposure to parabens by dermal penetration or by ingestion has been recently documented, and some parabens have demonstrated adverse effects on male reproduction in animal studies. However, human epidemiologic studies are lacking, according to (Canosa et al. 2006). Since (Harvey and Darbre 2004) have published a synthesis that exposure of the foetal / developing male to environmental oestrogens could be the cause of subsequent reproductive and developmental effects in men and also breast cancer in women, there has been little definitive research to provide conclusions to the hypothesis. This issue has been challenged by reviewing several researches on scientific databases.

(Bazin et al. 2010) studied the potential hazard of parabens. Following the EU EMEA (European Medicines Agency) Environmental Risk Assessment guideline for classification of dangerous substances and considering the results obtained with *Daphnia Magna*, methyl, ethyl and n-propyl parabens should be classified as harmful substances for aquatic organisms, whereas n-butyl and benzyl parabens as toxic substances. Concerning the biological activity, parabens are 8,000-900,000-fold less estrogenic than estradiol, the most estrogenic one being the aromatic compound benzylparaben. Higher estrogenic effect has been observed when estrogenic compounds have been added to parabens.

The different physico-chemical properties of the most used parabens have been given in table 1.

Table 1 physico-chemical properties of principal parabens (ELIE and Lionel 2007)

Structure générale				
	R	Methyl	Ethyl	Propyl
N° CAS	99-76-3	120-47-8	94-13-3	94-26-8
N° EINECS	202-785-7	204-399-4	202-307-7	202-318-7
N° INS/E (ester / sel)	E218 / E219	E214 / E215	E216 / E217	-
Masse Molaire (g.mol <sup>-1</sup> )	152,05	166,06	180,08	194,09
P <sub>f</sub> (°C)	131	116-118	96-98	68-69
P <sub>eb</sub> (°C)	270-280	297-298	-	-
Solubilité dans l'eau à 25°C	0,25 %	0,17 %	0,05 %	0,02 %
pKa	8,17	8,22	8,35	8,37
Apparence (à 25°C)	Cristaux incolores sans odeur et sans goût			

At ambient temperature, parabens are in crystal form, and so they should be solubilised in an adequate solvent for mixing in desired matrix. The solubility of parabens in water is very low. According to table 1, the solubility of Methyl, Ethyl, Propyl and butyl parabens are 0.25%, 0.17%, 0.05% and 0.02% respectively.

Cosmetics manufacturers and food processors use methyl and propyl parabens most extensively (Xiaoyun Ye et al. 2006).

**Propyl paraben** is a stable, non-volatile compound used as an antimicrobial preservative in foods, drugs and cosmetics for over 50 years. It is an ester of p-hydroxybenzoate. Propyl paraben is readily absorbed via the gastrointestinal tract and dermis. It is hydrolyzed to p-hydroxybenzoic acid, and then conjugated, and the conjugates are rapidly excreted in the urine. There is no evidence of accumulation. Acute toxicity studies in animals indicate that propyl paraben is relatively non-toxic by both oral and parenteral routes, although it is mildly irritating to the skin. Following chronic administration, no-observed-effect levels (NOEL) as high as 1200–4000 mg/kg have been reported and a no-observed-adverse-effect level (NOAEL) in the rat of 5500 mg/kg is posited. Propyl paraben is not carcinogenic, mutagenic or clastogenic. It is not cytogenic in vitro in the absence of carboxyesterase inhibitors. The mechanism of hydrolyzing and conjugation of propyl paraben may be linked to mitochondrial failure, dependent on induction of membrane permeability transition accompanied by the mitochondrial depolarization and depletion of cellular ATP through uncoupling of oxidative phosphorylation (Soni et al. 2001).

**Methyl paraben** is a methyl ester of p-hydroxybenzoic acid. As well as Propyl paraben, it is a stable, non-volatile compound used as an antimicrobial preservative in foods, drugs and cosmetics for over 50 years. Methyl paraben is readily and completely absorbed through the skin and from the gastrointestinal tract. It is hydrolyzed to p-hydroxybenzoic acid, conjugated, and the conjugates are rapidly excreted in the urine. There is no evidence of accumulation. Acute toxicity studies in animals indicate that methyl paraben is practically non-toxic by both oral and parenteral routes. In a population with normal skin, methyl paraben is practically non-irritating and non-sensitizing. In chronic administration studies, no-observed-effect levels (NOEL) as high as 1050 mg/kg have been reported and a no-observed-adverse-effect level (NOAEL) in the rat of 5700 mg/kg is posited.

Methyl paraben is not carcinogenic or mutagenic. It is not teratogenic or embryotoxic and is negative in the uterotrophic assay (Soni et al. 2002).

## 2. Human exposure to parabens

In humans, the metabolism of parabens may include the formation of conjugated species (e.g., glucuronides and sulfates), but the free species—not the conjugated forms—are considered biologically active. Therefore, information on the concentration of these free species in blood or urine could be helpful for risk assessment. Because conjugates could hydrolyze to their corresponding free forms during collection, handling, and storage of biological specimens, information on the temporal stability of the conjugates is of interest. In the study which was done by (Ye et al. 2009), the stability of the conjugates (glucuronides and sulphides) of four phenols — bisphenol A, benzophenone-3, triclosan, and 2,5-dichlorophenol — and two parabens — methyl paraben and propyl paraben — in 16 human serum samples for 30 days at above-freezing temperature storage conditions (4 °C, room temperature, and 37 °C) were investigated. They found that the percentage of the conjugated species of the four detected compounds (2,5-dichlorophenol, triclosan, and methyl and propyl parabens) in these serum specimens even when stored at 37 °C for at least 30 days did not vary significantly. These preliminary data suggest that the phenols' serum conjugates appear to be more stable than their corresponding urinary conjugates.

Recently, (Calafat et al. 2010) have assessed exposure to methyl, ethyl, propyl, and butyl parabens in a representative sample of persons  $\geq 6$  years of age in the U.S. general population from the 2005-2006 National Health and Nutrition Examination Survey. They analyzed 2,548 urine samples by using online solid-phase extraction coupled to isotope dilution-high-performance liquid chromatography/tandem mass spectrometry. They detected methyl paraben (MP) and propyl paraben (PP) in 99.1% and 92.7% of the samples, respectively. They also detected ethyl (42.4%) and butyl (47%) parabens less frequently and at median concentrations at least one order of magnitude lower than MP (63.5  $\mu\text{g/L}$ ) and PP (8.7  $\mu\text{g/L}$ ). Least-square geometric mean (LSGM) concentrations of MP were significantly higher ( $p \leq 0.01$ ) among non-Hispanic blacks than among non-Hispanic whites except at older ages ( $\geq 60$  years). Adolescent and adult females had significantly higher ( $p < 0.01$ ) LSGM concentrations of MP and PP than did adolescent and adult males. Females were more likely than males and non-Hispanic blacks were more likely than non-Hispanic whites to have concentrations above the 95th percentile. The general U.S. population was exposed to several parabens during 2005-2006. Differences in the urinary concentrations of MP and PP by sex and race/ethnicity likely reflect the use of personal care products containing these compounds.

(Janjua et al. 2008) have examined this hypothesis that humans can be exposed to butyl paraben (BP), diethyl phthalate (DEP) and dibutyl phthalate (DBP) through dermal absorption. They have investigated the urinary concentrations of BP and metabolites of DEP and DBP after topical application. In this study, in a 2-week single-blinded study, 26 healthy Caucasian male subjects were given a whole body topical application of basic cream 2  $\text{mg/cm}^2$  (control week) and then a cream containing 2% (w/w) of DEP, DBP and BP each (treatment week) daily. Then twenty-four-hour urine samples were collected. Urinary total, and unconjugated BP, monoethyl phthalate (MEP) and monobutyl phthalate (MBP) metabolites were analysed by Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS). All 26 subjects showed increased excretion of MEP, MBP and BP following topical application. Total MEP, MBP and BP (mean  $\pm$  SEM) excreted in urine in the treatment week were, respectively, 41  $\pm$  1.9, 11.8  $\pm$  0.6 and 2.6  $\pm$  0.1  $\text{mg/24 h}$ . On average 5.79, 1.82 and 0.32%, respectively, of the applied DEP, DBP and BP could be recovered in urine as

MEP, MBP and BP. In conclusion, it was revealed that the three chemicals are systemically absorbed, metabolized and excreted in urine following application on the skin in a cream preparation.

(Darbre and Harvey 2008) have reported that the presence of intact paraben esters (free parabens) in human body tissues has been confirmed by independent measurements in human urine, and the ability of parabens to penetrate human skin intact without breakdown by esterases and to be absorbed systemically has been demonstrated through studies not only in vitro but also in vivo using healthy human subjects.

(Xiaoyun Ye et al. 2006) assessed human exposure to parabens by measuring in urine the conjugated or free species of parabens or their metabolites. They evaluated the use of free and conjugated parent parabens as new biomarkers for human exposure to these compounds. They detected methyl and n-propyl parabens at the highest median concentrations (43.9 ng/mL and 9.05 ng/mL, respectively) in nearly all (> 96%) of the samples. The results, demonstrating the presence of urinary conjugates of parabens in humans, suggest that such conjugated parabens could be used as exposure biomarkers and finally this fact that conjugates appear to be the main urinary products of parabens and may be important for risk assessment.

### 3. Affect of parabens on human health

(Okubo et al. 2001) examined Estrogenic activities of the phenolic preservatives methylparaben, ethylparaben, propylparaben, butylparaben, isopropylparaben and isobutylparaben by assaying estrogen-receptor (ER)-dependent proliferation of MCF-7 cells. In conclusion, they achieved this result that parabens have ER-dependent estrogenic activities, and their effects on the intracellular signalling pathway might be different from that of 17beta-estradiol.

(Harvey and Darbre 2004) have verified two hypotheses. The first was that human exposure and low potency of environmental oestrogens may have reduced concerns and the second that chemicals applied in body care cosmetics (including moisturizers, creams, sprays or lotions applied to axilla, chest or breast areas) affecting breast cancer incidence in women presents a different case scenario, not least in the consideration of the exposure issues. The hypothesis for a link between oestrogenic ingredients in underarm and body care cosmetics and breast cancer was forwarded and reviewed in terms of: data on exposure to body care cosmetics and parabens, including dermal absorption; paraben oestrogenicity; the role of oestrogen in breast cancer; detection of parabens in breast tumours; recent epidemiology studies of underarm cosmetics use and breast cancer; the toxicology database; the current regulatory status of parabens and regulatory toxicology data uncertainties. They called for further research to provide the first evidence that environmental factors may be adversely affecting human health by endocrine disruption, because exposure to oestrogenic chemicals through application of body care products (unlike diffuse environmental chemical exposures) should be amenable to evaluation, quantification and control. The exposure issues are clear and the exposed population is large, and these factors should provide the necessary impetus to investigate this potential issue of public health.

(Pugazhendhi, Pope and Darbre 2005) addressed the question of whether p-hydroxybenzoic acid, the common metabolite of the alkyl esters of p-hydroxybenzoic acid (parabens), possesses oestrogenic activity in human breast cancer cell lines. It has reported that p-hydroxybenzoic acid possesses oestrogenic activity in a panel of assays in human breast cancer cell lines. They concluded that removal of the ester group from parabens does not abrogate its oestrogenic activity and that p-hydroxybenzoic acid can give oestrogenic responses in human breast cancer cells.

(Golden, Gandy and Vollmer 2005) has revealed some test of parabens to varying degrees that individual paraben compounds have weakly estrogenic activity in some in vitro screening tests. In this research, a comparative approach involving both dose and potency was used to assess whether in utero or adult exposure to parabens might be associated with adverse effects mediated via an estrogen-modulating mode of action. Based on these comparisons using worst-case assumptions pertaining to total daily exposures to parabens and dose/potency comparisons with both human and animal no-observed-effect levels (NOELs) and lowest-observed-effect levels (LOELs) for estrogen or diethylstilbestrol (DES), it is biologically implausible that parabens could increase the risk of any estrogen-mediated endpoint, including effects on the male reproductive tract or breast cancer. Additional analysis based on the concept of a hygiene-based margin of safety (HBMOS), a comparative approach for assessing the estrogen activities of weakly active endocrine-active chemicals (EACs), demonstrates that worst-case daily exposure to parabens would present substantially less risk relative to exposure to naturally occurring EACs in the diet such as the phytoestrogen daidzein.

(Darbre and Harvey 2008) firstly measured the intact esters of p-hydroxybenzoic acid (parabens) in human breast cancer tissues, and suggested that their presence in the human body might originate from topical application of bodycare cosmetics. With the continued use of parabens in the majority of bodycare cosmetics, there is a need to carry out detailed evaluation of the potential for parabens, together with other oestrogenic and genotoxic co-formulants of bodycare cosmetics, to increase female breast cancer incidence, to interfere with male reproductive functions and to influence development of malignant melanoma which has also recently been shown to be influenced by oestrogenic stimulation.

(Calafat et al. 2009) demonstrated that exposure to polyvinyl chloride plastic medical devices containing di(2-ethylhexyl) phthalate (DEHP) was associated with higher urinary concentrations of several DEHP metabolites in 54 premature infants in two neonatal intensive care units than in the general population. For 42 of these infants urinary concentrations of several phenols was evaluated, including bisphenol A (BPA), in association with the use of the same medical devices. They measured the urinary concentrations of free and total (free plus conjugated) species of BPA, triclosan, benzophenone-3, methyl paraben, and propyl paraben. The percentage of BPA present as its conjugated species was > 90% in more than three-quarters of the premature infants. Intensity of rise of products containing DEHP was strongly associated with BPA total concentrations but not with any other phenol. Adjusting for institution and sex, BPA total concentrations among infants in the group of high use of DEHP-containing products were 8.75 times as high as among infants in the low use group ( $p < 0.0001$ ). Similarly, after adjusting for sex and DEHP-containing product use category, BPA total concentrations among infants in Institution A were 16.6 times as high as those among infants in Institution B ( $p < 0.0001$ ). BPA geometric mean urinary concentration (30.3  $\mu\text{g/L}$ ) among premature infants undergoing intensive therapeutic medical interventions was one order of magnitude higher than that among the general population. Conjugated species were the primary urinary metabolites of BPA, suggesting that premature infants have some capacity to metabolize BPA. The differences in exposure to BPA by intensity of use of DEHP-containing medical products highlight the need for further studies to determine the specific source(s) of exposure to BPA.

Results presented by (Darbre and Charles 2010) and elsewhere demonstrate that in combination, chemicals can give oestrogenic responses at lower concentrations, which suggests that in the breast, low doses of many compounds could sum to give a significant oestrogenic stimulus. Updated incidence figures show a continued disproportionate incidence of breast cancer in Britain in the upper outer quadrant of the breast which is also the region to which multiple cosmetic chemicals are applied. They concluded that if exposure to complex mixtures of oestrogenic chemicals in consumer

products is a factor in breast cancer development, then a strategy for breast cancer prevention could become possible.

Many environmental compounds with oestrogenic activity are measurable in the human breast and oestrogen is a known factor in breast cancer development. Exposure to environmental oestrogens occurs through diet, household products and cosmetics, but concentrations of single compounds in breast tissue are generally lower than needed for assayable oestrogenic responses (Darbre and Charles 2010).

Relationships between urinary concentrations of parabens and markers of male reproductive health were investigated by (Meeker et al. 2011) in an ongoing reproductive epidemiology study. Urine samples collected from male partners attending an infertility clinic were analyzed for methyl paraben (MP), propyl paraben (PP), butyl paraben (BP), and bisphenol A (BPA). Associations with serum hormone levels (n = 167), semen quality parameters (n = 190), and sperm DNA damage measures (n = 132) were assessed using multivariable linear regression. No statistically significant associations were observed between MP or PP and the measured parameters. Urinary BP concentration were not associated with hormone levels or conventional semen quality parameters, but they were positively associated with sperm DNA damage (p for trend = 0.03). Assessment of paraben concentrations measured on repeated urine samples from a subset of the men (n = 78) revealed substantial temporal variability. Finally, no evidence was found for a relationship between urinary parabens and hormone levels or semen quality. However, the observation of a relationship between BP and sperm DNA damage warrants further investigation.

#### 4. Affect of parabens on animal health

(Nakagawa and Moldéus 1998) studied the relationship between the metabolism and the cytotoxic effects of the alkyl esters of p-hydroxybenzoic acid (parabens) in freshly isolated rat hepatocytes. Incubation of hepatocytes with propyl-paraben (0.5 to 2.0 mM) elicited a concentration- and time-dependent cell death that was enhanced when enzymatic hydrolysis of propyl-paraben to p-hydroxybenzoic acid was inhibited by a carboxylesterase inhibitor, diazinon. In the comparative toxic effects based on cell viability, ATP level, and rhodamine 123 retention, butyl- and isobutyl-parabens were more toxic than propyl- and isopropyl-parabens, and ethyl- and methyl-parabens and p-hydroxybenzoic acid were less toxic than propyl-paraben. These results indicate that a) propyl-paraben-induced cytotoxicity is mediated by the parent compound rather than by its metabolite p-hydroxybenzoic acid; b) the toxicity is associated with ATP depletion via impairment of mitochondrial function related to membrane potential and/or oxidative phosphorylation; and c) the toxic potency of parabens to hepatocytes or mitochondria depends on the relative elongation of alkyl side-chains esterified to the carboxyl group of p-hydroxybenzoic acid.

The oestrogenic activity of the parabens, methyl-, ethyl- and propyl p-hydroxybenzoate, widely used as antimicrobials in food, and butyl p-hydroxybenzoate, which is used in cosmetic products, and their shared main metabolite p-hydroxybenzoic acid was investigated by (Hossaini, Larsen and Larsen 2000) in a mouse uterotrophic assay. p-Hydroxybenzoic acid and butyl p-hydroxybenzoate were also tested by the subcutaneous route in a rat uterotrophic assay. A significant increase in the uterus weight at day 4 was considered an oestrogenic effect. In the mouse assay, none of the compounds tested produced any oestrogenic response at dose levels up to 100 mg/kg body weight per day, for ethyl p-hydroxybenzoate even at a dose level of 1000 mg/kg body weight per day. In immature Wistar rats, subcutaneous administration of butyl p-hydroxybenzoate produced a weak oestrogenic response at 600 mg/kg body weight per day.

p-hydroxybenzoic acid ester compounds (parabens) exert a weak estrogenic activity as determined by *in vitro* estrogen receptor assay and *in vivo* uterotrophic assay. In the first study of (Oishi 2001), it was demonstrated that exposure of post-weaning mammals to butyl paraben adversely affects the secretion of testosterone and the function of the male reproductive system. In the last study of (Oishi 2002), it was shown that propyl paraben also adversely affects the hormonal secretion and the male reproductive functions. Propyl paraben was administered to 3-week-old rats which were divided into four groups of eight animals each, at doses of 0.00, 0.01, 0.10 and 1.00% with the AIN93G modified diet. At the end of 4 weeks, the rats were sacrificed by decapitation and the weights of testes, epididymides, prostates, seminal vesicles and preputial glands were determined. There were no treatment-related effects of propyl paraben on the organ weights in any of the study groups. Daily sperm production and its efficiency in the testis of all groups receiving propyl paraben significantly decreased. The serum testosterone concentration decreased in a dose-dependent manner and the decrease was significant in the group that received the highest dose. The exposure level at which this effect was observed is the same as the upper-limit acceptable daily intake (10 mg/kg body weight/day) of parabens in the European Community and Japan.

In a study by (Masten 2005) for U.S Department of Health and Human Services, the toxically effects of butylparaben as a widely used antioxidant and preservative in foods, pharmaceuticals, and cosmetics, was investigated. The results show that ingested butylparaben is rapidly absorbed from the gastrointestinal (GI) tract, metabolized, and excreted in the urine. Large doses, however, may cause irritation to the GI tract. In mice, rats, rabbits, and dogs, butylparaben was reported to be practically nontoxic. Results from one chronic feeding study in mice showed that butylparaben caused a high incidence of amyloidosis, affecting the spleen, liver, kidney, and/or adrenal gland. Reproductive studies in mice and rats suggested that maternal exposure to butylparaben in the diet results in adverse effects on the reproductive system of F1 male offspring. Butylparaben was not mutagenic in several short-term bioassays (e.g., Ames test, Chinese hamster ovary cells, and comet assay) and was reported to be non-carcinogenic in rats and mice.

The acute immobilization toxicity of benzoic acids substituted with hydroxyl and/or methoxyl groups on the aromatic ring was determined by (Kamaya, Fukaya and Suzuki 2005) for the freshwater crustacean *Daphnia magna* under neutralized condition (initial pH:  $7.45 \pm 0.05$ ). Toxicity, expressed as EC50 value, varied depending largely on the number and position of phenolic hydroxyl groups. Especially, benzoic acids with ortho-substituted hydroxyl groups were more toxic than benzoic acids with meta- and/or para-substituted hydroxyl groups. Whereas the limited data indicated that methoxyl substitution had relatively small and variable effects on the toxicity.

(Kawaguchi et al. 2010) studied high comparative binding affinity of isobutyl-paraben (IBP) to estrogen receptors. They examined the effects of maternal exposure of rats to IBP on plasma hormone concentrations and organ weights in dams, ratio of male Pups, anogenital distance, organ weights and plasma hormone concentrations in offspring, puberty, estrous cycle and response of organ weight and plasma hormone concentrations to estrogen in adult female offspring, and reproductive and adrenal function in adult male offspring, all of which are under developmental estrogen regulation, to clarify the estrogenic effects of IBP during gestation and lactation on the endocrine systems of dams and offspring. While maternal exposure of IBP decreased the plasma corticosterone concentration and increased the uterus weight in dams and increased uterine sensitivity to estrogen in adult female offspring, the other indices examined were largely unaffected by the present treatment. Even though these results indicate little sign of endocrine disrupting effects for IBP, the existence of activity may be a matter of concern due to the possible impact on the health of Future generations.

In another study, (Kawaguchi et al. 2009b) analyzed the effects of maternal IBP treatment on the emotional behaviour and learning performance in mature offspring Sprague-Dawley rats. Pregnant female ones were treated with IBP via a subcutaneous Silastic capsule. Consequently, the offspring were exposed to IBP during gestation through the placenta, and before weaning through the milk. Male and female offspring were tested for emotional behavior in an open field and in an elevated plus maze at five and six weeks old, respectively. IBP-exposed male (but not female) rats spent less time in the open arms of the elevated plus maze. At 11 weeks old, all females were gonadectomized and treated chronically with 17 beta-estradiol or cholesterol by Silastic capsules; all males were kept intact. They were tested for learning performance in a passive avoidance test and a Morris water maze. IBP exposure impaired the performance of males in the passive avoidance test. These findings suggest that male rats are more affected by early exposure to IBP than female rats. IBP affects their adult behaviour including anxiety and learning abilities.

From two last studies of (Kawaguchi et al. 2009b), Isobutyl-paraben (IBP), a widely used preservative, exhibits estrogenic activity. The effects of exposure to IBP during gestation and lactation via dam on social recognition behavior in ovariectomized offspring of Sprague-Dawley rats was analysed by (Kawaguchi et al. 2009a). Offspring were ovariectomized at 7 weeks of age, and were used in a social recognition test at 16 weeks of age. Each offspring was exposed to a novel ovariectomized rat four times and to a second novel rat in a fifth exposure. The IBP-exposed rats showed impaired social behaviour compared with controls. These data imply that early exposure to IBP may have an effect on adult social behaviour, which is reported to be an autism spectrum disorders in humans.

## 5. Fate and metabolite of parabens in environment

(Canosa et al. 2006) investigated chemical transformations of four alkyl esters of p-hydroxybenzoic acid, parabens, in chlorinated water samples. Quantification limits of parent species and their possible transformation products were at the low  $\text{ng L}^{-1}$  level. By employing ultrapure water solutions, for the first type of sample, only two by-products were detected for each paraben. They corresponded to chlorination of the aromatic ring in one or two carbons situated in ortho-positions to the hydroxyl group. Both species were also generated after the addition of parabens to chlorinated tap water. Moreover, three new transformation products were noticed for each parent compound. They were identified as bromo- and bromochloro-parabens, formed due to the existence of traces of bromide in tap water sources. In addition, the presence of the di-chlorinated forms of methyl and propyl paraben has been detected in raw sewage water samples.

(Guadarrama et al. 2008) by using a mathematical model studied four parabens (methyl, n-butyl, benzyl and isobutylparaben) in order to evaluate their estrogenic activity through simplified models. According with the calculated results, methylparaben is the paraben with higher estrogenic activity. The antibacterial activity of parabens was also explored considering the formation of potassium salts in the phenolic OH groups. From the obtained relative energy values, methylparaben is the most active preservative.

Nicotinamide is a hydrophilic molecule, freely soluble in water, used as cosmetic active ingredient for its moisturizing and depigmenting properties. Moreover it has the ability to augment the solubility of poorly water-soluble molecules acting as a hydrotrope. The aim of this work of (Nicoli et al. 2008) was to study the effect of nicotinamide on the transdermal permeation of methyl, ethyl, propyl and butyl paraben. Parabens flux was measured in vitro in the presence and absence of different amounts of nicotinamide. From solubility studies it was found that nicotinamide forms one or more complexes with methyl, propyl and butyl paraben in water, even though with low stability constants. The association of nicotinamide with parabens causes a

significant reduction of the permeability coefficients of these preservatives through rabbit ear skin, caused by a reduction of the stratum corneum/vehicle partition coefficient. The effects of nicotinamide on parabens solubility, permeation and partitioning are potentially very interesting because nicotinamide can facilitate paraben dissolution in aqueous media (solutions, gels), reduce parabens partitioning in the oily phase thus guaranteeing an effective concentration in the water phase in emulsion and reduce transdermal penetration, thus reducing the toxicological risk.

(Rudel and Perovich 2009) summarized reported indoor and outdoor air concentrations, chemical use and sources, and toxicity data for some chemical classes. These products include PCBs, chlorinated and brominated flame retardants, pesticides; used to control insects, weeds and other pests in agriculture, lawn maintenance, and the built environment; phthalates, plastics, fragrances, and also alkylphenols and parabens; used to preserve products like lotions and sunscreens. The median indoor air concentration of methyl paraben was 2.9 ng/m<sup>3</sup> and the maximum was 21 ng/m<sup>3</sup>. Small on-site greywater treatment and reuse plants are susceptible to high short-term variation in flow and pollutant concentrations and the flow ranges from no-flow periods to high-flow periods.

(Tay, Rahman and Abas 2010) investigated the reaction kinetics and degradation mechanism of parabens (methylparaben, ethylparaben, propylparaben and butylparaben) during ozonation. Hydroxylation has been found to be the major reaction formation. Through the hydroxylation reaction, a variety of hydroxylated parabens was formed.

(Brausch and Rand 2011) examined the acute and chronic toxicity data available for personal care products and highlights areas of concern. Personal care products including triclosan, paraben preservatives, and UV filters have evidence suggesting endocrine effects in aquatic organisms and thus need to be investigated and incorporated in definitive risk assessments. Finally, they noticed that published literature for environmental concentrations is fairly substantial for some PCP compounds (ex. TCS, DEET, fragrances) but relatively little is available for others (ex. TCC, UV filters, preservatives). Additional research for surface water concentrations of PCP compounds and uptake into biota needs to be conducted in order to develop aquatic risk assessments for PCPs released in WWTP effluent. There are also substantial data gaps in published literature addressing potential toxicity of PCPs to aquatic organisms.

(Johnson et al. 1999) examined the effects of bioavailability on degradation of 14C-p hydroxybenzoate using sterile soil inoculated with *Arthrobacter* sp. The results showed that *arthrobacter* sp. accessed 94% of p-hydroxybenzoate in soil.

## 6. Parabens in wastewaters

(Canosa et al. 2006) reported that in the wastewater samples taken in the Northwest of Spain, methyl and propyl parabens displaying the highest concentrations. On the other hand, benzyl paraben was never detected and they have also identified and quantified some chlorinated by-products of methyl and propyl paraben. Also, p-hydroxybenzoic acid was present in raw wastewater.

In France within the framework of AMPERES project (Cemagref 2006-2009) 21 French wastewater treatment plants were studied in order to measure the composition of raw and treated wastewaters regarding pharmaceutical substances. In the list of analysed substances there are not parabens. They noticed that compounds like antibiotics and betablockers are not efficiently removed unless specific tertiary treatments are used, such as activated carbon filtration, reverse osmosis and ozonation.

(Eriksson et al. 2009) measured the concentration of parabens in influant and effluent of small on-site greywater treatment and reuse plants in Denmark. They noticed that paraben degradation was proven to occur in the rotating biological contactor (RBC) by the biofilm which contains microorganisms. In the greywater samples which had not passed by RBC, all the parabens were present until the end of the experiment, illustrating that any bacteria suspended in the greywater did not degrade parabens..

(Yamamoto et al. 2007) in Japan, (Jonkers et al. 2009) in Switzerland, reported the removal efficiency for parabens of batch-activated sludge treatment and chlorination as a tertiary treatment in WWTPs are to 99% and 72% respectively.

(Chris 2010) classified the parabens as “NOT Well Studied” microcontaminant in wastewater treatment plants in Canada.

## 7. Important viewpoints

- Human and animals expose to parabens by dermal penetration and/or by ingestion.
- Parabens have been considered harmful (estrogenic and toxic activity) for health and environment by several individual researches but there are not epidemiologic evidences.
- In human body, free parabens are absorbed, metabolised (Hydrolysed and conjugated) and finally excreted in urine. Conjugates are the main urinary product of parabens.
- Parabens existing in household product void in free and/or hydrolysed state in environment.
- So parabens as an emerging pollutant is found in domestic wastewaters.
- Degradation of parabens in wastewater by chlorination, ozonation and biological methods (adding the bacteria) is done in WWTPs as tertiary treatment (Artificial).
- The fate and transfer of parabens by individual domestic wastewater treatment systems and its impact on soil have not been studied (lack of research)

## 8. Perspective for ANCREs project

In human body, parabens are mainly hydrolyzed to p-hydroxybenzoic acid, which can then be conjugated with glycine, glucuronide, and sulfate and excreted in urine (Ye et al. 2006). So the free and conjugated parabens can be found in wastewater and therefore, it should applied a methodology to detect firstly the both, free parabens and also the conjugated parabens to find the total paraben species (free and conjugated metabolites) in soil water sample. In the methodology which will be applied for detecting parabens, it should search for parent/individual parabens (conjugated or free species) in soil water samples. In this way, the methodology which has been developed for detecting the parabens in urine samples by (Ye et al. 2006) [The author believes that the methodology can be used for water sample by some modifications] can be applied for soil water samples. The procedure for extracting the deconjugated parabens from the soil water samples involved concurrent online SPEHPLC operation with peak focusing which has been explained in the methodology of Ye et al. (2006). To determine the concentrations of the free species ( $C_{free}$ ), we followed the above procedure without the enzymatic hydrolysis treatment.

After literatures, less attention has been paid to the reactivity of personal care products when mixed with tap water in our homes. Tap water is normally amended with free chlorine to insure its bacteriological quality throughout the distribution system. Chlorine is a potent oxidant able to react with natural organic matter and anthropogenic chemicals rendering different halogenated by-

products. Some of them are toxic species, which might suppose a potential risk for human health. Particularly, compounds containing phenolic groups in their structures show favourable chlorination kinetics (Canosa et al. 2006).

Parabens can easily react with free chlorine when mixed with chlorinated tap water, yielding mostly mono and dichlorinated derivatives that, in fact, have already been detected in raw sewage water. Chlorinated by-products seem to be more persistent than parent species during biodegradation experiments (Cela et al. 2010a).

Although this halogenation masks the apparent estrogenic activity of the parent compounds, the resulting chlorinated by-products show higher toxicity responses when working with *Daphnia magna* bioassays (Terasaki, Makino and Tatarazako 2009).

So, they should be included in any ecological or environmental study involving parabens. In this way, an assessment of the accuracy and environmental biodegradability of parabens and halogenated derivatives of methyl and propyl paraben are necessary. The samples will process following a solid phase extraction (SPE) method and analysed by liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) (Cela et al. 2010a).

The optimized and validated methods, based on GC-MS and LC-MS/MS, for the determination of parabens and their chlorinated by-products in raw and treated wastewater as well as in surface water has been developed in Institute for Food Analysis and Research in Spain. These procedures render lower limits of quantification LOQs in the low ng/L, which, sometimes are controlled by procedural blanks. This methodology for measurement has been explained in detail by (Gonzalez-Marino et al. 2009) and it can be applied in ANCREs project for the soil water samples. In this method, the conventional filters used in laboratory have a size of 0.45 micron and also all data published for wastewater samples correspond to filtered samples, So the soil water sampler with pore sizes bigger than 0.45 micron can well collected the water which containing parabens without any clogging.

The results of the current studies of the researchers in Institute for Food Analysis and Research in Spain who used the (LC/TOF/MS) (Ferrer and Thurman 2002) method show that all paraben compounds (parabens and halogenated by-products) were easily degraded. And that is because by using the LC-TOF-MS method as determination technique, they were unable to identify any decomposition by-product, so the preliminary conclusion is that the parabens are mineralized. This result can be challenged in ANCREs projects. For evaluating the fate of parabens in soil after infiltration the treated wastewater and finding the role of soil against parabens, the different levels of soil water will be analysed by LC-TOF-MS method.

Finally the method which was developed by (Cela et al. 2010b) can be employed to determine the simultaneous presence of triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) and seven parabens (esters of 4-hydroxybenzoic acid), including methyl, ethyl, i-propyl, n-propyl, i-butyl, n-butyl and benzyl groups in different water samples .

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