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Exposure to UV filters during summer and winter in Danish kindergarten children

Marianna Krause, Anna-Maria Andersson, Niels E. Skakkebaek, Hanne Frederiksen *

Dept. of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark
International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, University of Copenhagen, Denmark

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ABSTRACT

Background: Ultra violet (UV) filters with known or suspected endocrine disrupting properties are widely used in sunscreens and other personal care products, clothing, food packaging and many other consumer products. Danish kindergarten children have sunscreens applied daily during summer to prevent skin burns.
Objectives: To estimate the assumed contribution of sunscreens to the total exposure to UV filters, we measured the urinary excretion of UV filters during summer and winter in kindergarten children.
Methods: Spot- and first morning urines were collected during a summer and a winter day in 2013. A total of 266 urine samples were collected from 55 children and were analysed for content of benzophenone (BP), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), 5-chloro-2-hydroxybenzophenone (BP-7), 4-methyl-benzophenone (4-MBP), 4-hydroxybenzophenone (4-HBP), 3-(4-methylbenzylidene)-camphor (4-MBC), and 3-benzylidene camphor (3-BC) by LC-MS/MS.
Results: Of the analysed UV filters, the children excreted predominantly BP-1, BP-3 and 4-HBP. The urine levels were significantly higher in summer samples compared to winter samples, however exposure during winter was still evident. Furthermore, children with the highest concentrations of UV filters in summer urines also tended to be among those with the highest winter levels.
Conclusion: Exposures to UV filters during summertime can partly be explained by the intended use of UV filters in sunscreens, which is considered to be beneficial for children during outdoor activities. However, exposure to UV filters all year round together with large inter-individual variation indicate that children's exposure to UV filters also comes from other consumer items, presumably highly influenced by the general lifestyle of an individual child: this is completely unintended, without benefit, and potentially harmful.

1. Introduction

Ultra violet (UV) filters are a group of chemicals that protect our skin from UV light. Due to their UV protecting properties they constitute the active ingredients of sunscreens. Furthermore, UV-filters counteract photo-oxidation and thereby protect plastic and other industrial products from becoming fragile and friable in the sun. Some of them also have the property to absorb UV-light and therefore protect coloured dyes in paints and textiles from bleaching. Due to these properties they are widely used in other personal care products in addition to cloths, furniture, food packaging, washing powder and many other consumer items (The Danish Enviromental Protection Agency, 2015).

Human biomonitoring studies have confirmed widespread human exposure to certain UV filters. Benzophenone-3 (BP-3[1]) was detected in almost all (80–96%) urine samples collected from the general population in the US (Calafat et al., 2008); from pregnant women in France (Philippat et al., 2011; Wolff et al., 2008); from Danish children, adolescents, young men and pregnant women (Frederiksen et al., 2014); from mothers and their children in Denmark (Frederiksen et al., 2013b); and has also been detected in human breast milk in Switzerland (Schlumpf et al., 2010). Levels of UV filters in samples collected in November in

* Corresponding author.
E-mail address: Hanne.Frederiksen@regionh.dk (H. Frederiksen).

[1] Abbreviations: BP: benzophenone; BP-1: benzophenone-1; BP-2: benzophenone-2; BP-3: benzophenone-3; BP-7: 5 choloro-2-hydroxybenzophenone; BP-8: benzophenone-8; bw: body weight; LC-MS/MS: liquid chromatography mass spectrometry; LOD: limit of detection; NHANES: National Health and Nutrition Examination Survey; NOAEL: no observed adverse effect levels; RSD: relative standard deviation; THB: 2,3,4-trihydroxybenzophenone; 3-BC: 3-benzylidene camphor; 4-HBP: 4-hydroxy-benzophenone; 4-MBC: 3-(4-methylbenzylidene)-camphor; 4-MBP: 4-methyl-benzophenone.

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Denmark, when use of sunscreen is very unlikely, indicate exposure throughout the year regardless of use of sunscreen (Frederiksen et al., 2013a).

An interventional study from our group has previously shown that UV filters can be detected in serum and urine of adult volunteers shortly after dermal application, proving that they can pass through the skin and into the body (Janjua et al., 2008).

A wide range of *in vitro* and *in vivo* studies have shown endocrine disrupting properties of some UV filters. They are reported to exhibit oestrogenic and antiandrogenic properties, as well as disturb thyroid function, and affect puberty, growth, development of genital organs and semen quality (for review see (Krause et al., 2012)).

Daily topical application of sunscreen for the prevention of sunburn is a normal procedure in Danish kindergartens from April to September following the recommendations from The Danish Health and Medicines Authority and The Danish Cancer Society (Danish cancer society, 2013; Danish Health and Medicines Authority, 2014). Furthermore, kindergartens ask parents to apply sunscreens in the morning in order to supplement the subsequent application in kindergarten. Danish kindergarten children 3–5 years of age might, therefore, be the most exposed population during summer time.

However, because of the possibility of being exposed to UV filters during winter from plastics and from personal care and other products, we explored the seasonal variation in the exposure to UV filters in Danish kindergarten children. It is assumed that sunscreens applied during summer are the major source of the exposure to UV filters, however, we measured urinary levels of 9 different UV filters in samples from kindergarten children, collected during a day in summer and in winter in 2013.

2. Methods

2.1. Study participants

55 kindergarten children aged 3–5 years from two different kindergartens in Copenhagen, Denmark, participated in the study in 2013. Only children whose parents agreed for participation by written consent were included in the study (44%). Children who were still using diapers or were expected to leave kindergarten to start school before our winter visit were excluded from the study. Otherwise children could participate regardless of their presence at both or only at one of the two urine collection days. To avoid any interference with their usual life style, parents and kindergarten staff were informed that urine samples would be collected for the measurement of different endocrine disrupting chemicals, and not specifically for UV filters. Furthermore, the information about sunscreen application at home and at kindergarten was obtained at the end of the day when urine samples were collected. The visits at the kindergartens for collection of urine samples were carried out on one sunny summer day and again on a winter day for each of the two kindergartens. Neither children nor parents were given prior information about the exact date for urine sampling in order to avoid bias in their behaviour these days.

The study was approved by The Danish Ethical committee (Protocol nr.: H-1-2013-019) and The Danish Data Protection Agency Journal nr. 02841/30-1146.

2.2. Spot urine samples

During the days of the visits to the kindergartens, a team of research assistants conducted the urine sampling on site from just before lunch time and throughout the afternoon (10:10 a.m. to 4:00 p.m). Urine was collected whenever a child felt like urinating and allowed one of the assistants to collect the urine. For the majority of the children, the first spot urine sample was collected between 10:10 a.m. and 1 p.m. Each child subsequently delivered as many urine samples during the day as they wanted resulting in 1 to 8 samples per child. Spot urine was collected in polypropylene cups. Immediately after collection, urine samples were decanted into 20 ml scintillation vials and kept cold in a dark cool box with refrigerating units until being transported to the laboratory at the end of the day. In the laboratory, all scintillation vials were covered with aluminium foil to protect samples from daylight before storage at −20 °C until further analysis. All material and lab ware for urine collection and storage were previously tested and confirmed negative for contamination with UV filters.

A total of 188 spot urine samples were collected from 55 children (for details please see supplementary Table).

2.3. First morning urine samples

At each day of our visit when the children were picked up by their parents in the afternoon, the parents received a kit for collection of next day's first morning urine. The kit contained instruction about morning urine collection and a polypropylene cup for collection of first morning urine, and a polyethylene bottle. Parents were asked to decant the urine from cup to bottle and bring the sample to the kindergarten in the same morning that it was collected. At the kindergarten, the first morning urine samples were kept cold in a dark cool box with refrigerating units and the same procedure for storage as for the spot urine was followed. In total, 78 first morning urine samples were collected from 55 children. One morning urine sample was subsequently excluded due to a missing label.

2.4. Personal characteristics

Weight and height were measured on site in the kindergarten by our staff on the day for spot urine sampling both in summer and in winter.

The normal procedure in Denmark is that parents are asked to apply sunscreen on their child every day from April to September in the morning before delivering their child to the kindergarten. In this way, it only takes a short time for the kindergarten staff to supplement the sunscreen application in the middle of the day. In addition to the kit for the first morning urine collection, the parents also received a brief questionnaire. Besides information about name, age and gender, the questionnaire also included the question “Did you apply sunscreen to your child at home on the day the urine samples were collected at the kindergarten?” The completed questionnaires were collected, together with the first morning urine samples, on the day after spot urine collection in kindergartens. 65% of the participating children had sunscreen applied at home before collection of summer urine samples. Information from the questionnaire confirms that none of the children had sunscreen applied prior to winter samples collection. Information about sunscreen use at home prior to winter sampling was missing from only 6 out of 61 children, but the use of sunscreen during winter time in Denmark is considered to be very unlikely. In order to avoid interference with the usual working practices of kindergartens staff, we did not register the application of the sunscreen for each child during the day but we observed that it was a normal procedure in both kindergartens to apply sunscreens on children after lunch before outdoor activities. The information about sunscreen application was partly recalled from staff's memories at the end of the day and partly collected from their own lists. On the day of summer urine collection, 53% of the children had sunscreen applied at the kindergarten of whom 29% have previously had sunscreen applied at home. Only 11% of children did not have any sunscreen applied either at home or at the kindergarten on the day of summer urine collection. Sunscreens are not applied in kindergartens during wintertime in Denmark.

2.5. Chemical analysis

All chemical analyses for simultaneous determination of total (free and conjugated) content of nine different UV filters (benzophenone (BP), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3), 5-chloro-2-hydroxybenzophenone (BP-7), 4-hydroxy-

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