TRICLOSAN AND BENZOPHENONE-3 IN AUSTRALIAN POOLED INFANT URINE

Heffernan AL[1]*, Eaglesham G[1], Hobson P[2], Sly P[3], Toms LM[4], Mueller J[1]

[1]National Research Centre for Environmental Toxicology, University of Queensland, 39 Kessels Rd, Coopers Plains, Queensland, Australia 4108; [2]Sullivan Nicolaides Pathology, Cnr Whitmore St & Seven Oaks St Taringa, Queensland, Australia 4068; [3]Children's Health and Environment Program, Queensland Children's Medical Research Institute, University of Queensland, Herston Rd, Herston, Queensland, Australia 4029; [4]Queensland University of Technology, School of Clinical Sciences and Institute of Health and Biomedical Innovation, Faculty of Health, 2 George St, Brisbane, Queensland, Australia 4000.

Introduction

The period of developmental vulnerability begins at conception and extends through gestation, parturition, infanthood and adolescence. The World Health Organisation (WHO) acknowledges that children experience quantitatively and qualitatively different exposures to chemicals than adults, and that children may be more or less sensitive to a chemical than adults [1, 2]. For instance, because of mouthing behaviours, children have higher exposure to chemicals through non-dietary ingestion than adults [3, 4], and the possibility exists for different metabolism and/or toxicity between different groups due to the immaturity of defense mechanisms that are fully developed in adults [1]. Traditional toxicological studies are inappropriate for assessing the results of exposure at very low levels during critical periods of development. Biomonitoring data can be used to identify where policies should be directed in order to reduce exposure.

Benzophenone-3 (BP-3, or oxybenzone) is a broadband ultraviolet (UV) radiation filter used as a sunscreen and photostabiliser in various cosmetic products worldwide. Human health effects from chronic, low-dose exposure are unknown, although photosensitivity and eczema following dermal application has been reported [5, 6].

Triclosan (TCS) is a synthetic, broad spectrum antimicrobial agent used in a wide range of personal care products, including toothpaste, soaps, and deodorants; as well as being impregnated in some kitchen utensils and children's toys[7]. Exposure for the general population is through dermal application or oral use of products containing TCS. TCS is rapidly metabolized and excreted from the human body. Human data provides no evidence for genotoxicity or carcinogenicity, although it is a known respiratory and dermal irritant [8]. The concern is the potential for the development of TCS-resistant pathogens because of its widespread use [9].

The overall objective of this study was to evaluate the exposure of Australian infants to ubiquitous contaminants TCS and BP-3, thereby establishing baseline values in Australia, and to compare these values to the results from other population surveys globally.

Materials and methods

Samples: De-identified urine samples were obtained from Sullivan Nicolaides Pathology (Taringa, Queensland, Australia) from surplus stored specimens that had been collected as part of routine pathology testing. Prior to pooling, samples were stratified according to age and sex. The age groups were as follows: 0-<0.5, ≥0.5-<1, ≥1-<1.5, ≥1.5-<2, ≥2-<2.5, ≥2.5-<3, ≥3-<3.5, ≥3.5-<4, ≥4-<4.5, ≥4.5-<5years. 42 pools were created from 294 individual samples, where each pool contained 7 samples, with replicate pools per strata where sufficient samples were available. Adult pools were stratified in larger age brackets, with 28 donors per pool as follows: ≥15-<30, ≥30-<45, ≥45-<60, ≥60-<75 years, (9 pools from 252 individual samples). Date of birth, date of sample collection, and sex were known for each sample. The mean age of the pool was calculated by taking the mean of all ages of donors included in the pool.

Analysis: Analysis of urine samples for TCS and BP-3 was based on a method described previously [10]. Briefly, 100uL urine was diluted to 1mL using HPLC-grade milli-Q water, deconjugated using β-glucuronidase (*Helix pomatia* H1) and incubated at 37°C for 8 hours. Samples were injected directly using a Gilson GX-271 liquid handler, and analysed using online solid phase extraction (SPE). The liquid chromatography tandem mass spectrometry (LC-MS/MS) system consisted of a Shimadzu Prominence UFLC coupled to an AB Sciex API 5500 QTRAP equipped with an APCI (atmospheric pressure chemical ionisation) source. Chromatographic separation was achieved using a Syneri Fusion 50×2.00mm, 2.5µm column. For some samples, concentrations of target analytes were beyond linear range of calibration curve. These samples were analysed using direct injection (i.e. no SPE) with the same chromatographic profile. Quantitation was performed using isotope dilution.

Quality control/quality assurance:
Samples were analysed in multiple batches, with blank levels and limits of reporting (LOR), respectively of <LOD and 0.5ng/ml for TCS, and 3.2 and 10ng/ml for BP-3 (three times the concentration of the blanks). No blank subtraction was performed.

Results and Discussion
Although individual samples were stratified by age and sex prior to pooling, samples were not differentiated according to sex for the purposes of this study, because the female data set was incomplete (only 16 of 40 pools analysed at time of publication).

Benzophenone-3: BP-3 was detected in all 51samples, with an average concentration of 116ng/ml urine, and a range of 20.6-1750ng/ml for infants and 23.3-68.0ng/ml for adults. Figure 1 shows urinary concentration versus average age, where each data point represents a single pooled sample (with 7 individuals per pool for <5years, and 28 individuals per pool for ≥15-<75years).

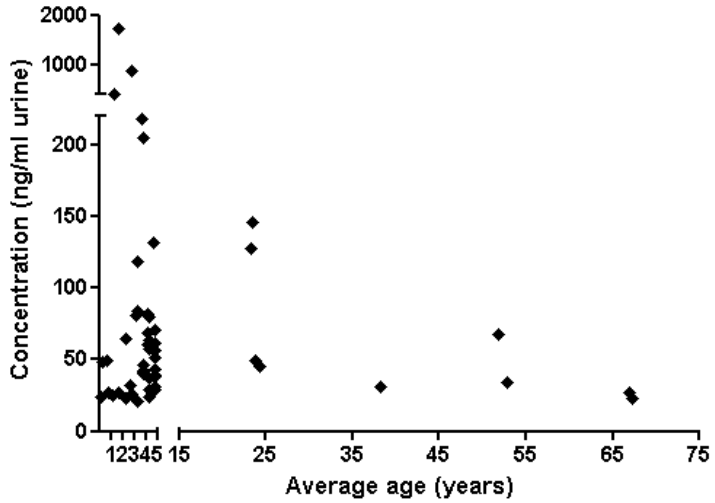

Figure 1: Concentration of BP-3 in pooled urine samples from children (0-<5 years) and adults (≥15-<75 years) (ng/ml urine)

It appears that the concentration of BP-3 increases from approximately the age of two to four years, after which the results are scattered. This age range corresponds with the typical age at which children start to attend day care centres. Interestingly, the apparent increase in urinary concentration is seen beyond two years of age, and the European Commission recommends not to use BP-3-containing products on infants younger than two years as their metabolic processes may be insufficient to process BP-3 and its breakdown products [5].

The United States Centres for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) presents health-related data for 212 chemicals for a nationally-representative percentage of the population. The average level of BP-3 from the 2007-08 cohort was 18.3ng/ml, with a 95th percentile of 801ng/ml (non creatinine corrected) [11]. The average level in Australian infants is more than 5-fold higher than the NHANES data set.

It should be noted that the data set is biased in that the pools were created from surplus routine pathology urine samples i.e. from non-healthy individuals. The samples were most frequently from individuals suffering from urinary tract infections (Peter Hobson, personal communication), in which case their behaviours may have been more reserved than normal, for example, less recreation time outdoors (requiring sunscreen). In this instance, the urinary concentrations measured in these samples may actually underestimate the levels in general Australian population.

Triclosan: TCS was detected in 94% samples, with an average concentration of 58.9ng/ml. The concentration ranged from <LOD-449ng/ml for infants, and 47.7-217ng/ml for adults, with the results shown in Figure 2, where each data point represents a single pooled sample (with 7 individuals per pool for <5years, and 28 individuals per pool for ≥15-<75years).

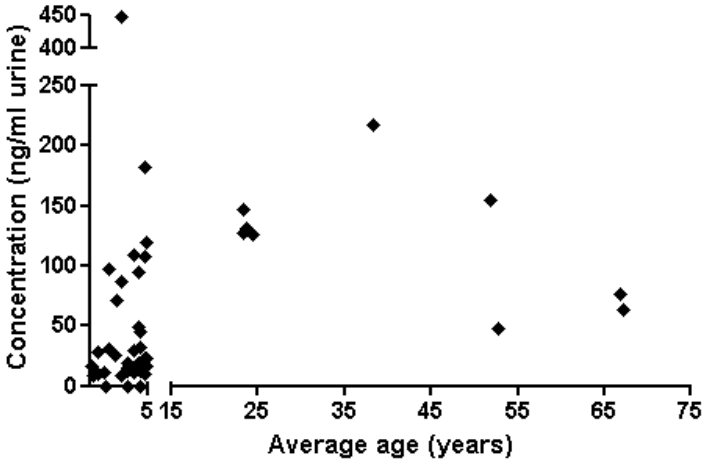

Figure 2: Concentration of triclosan (conjugated and un-conjugated) in pooled urine samples from children (0-<5 years) and adults (≥15-<75 years) (ng/ml urine)

Although the data shows no clear age trends, the levels in the Australian population are more than double those reported by NHANES (compare average 15.4ng/ml for American general population [11], with 58.9ng/ml for the Australian population).

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