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Blood concentrations of polycyclic musks in healthy young adults

H.-P. Hutter [a,*], P. Wallner [b], H. Moshammer [a], W. Hartl [c], R. Sattelberger [c], G. Lorbeer [c], M. Kundi [a]

[a] *Institute of Environmental Health, Center for Public Health, Medical University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria*

[b] *Medicine and Environmental Protection, Vienna, Austria*

[c] *Federal Environmental Agency, Vienna, Austria*

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Abstract

Knowledge on the concentration of polycyclic musk fragrance compounds in human blood is sparse. This study examined the concentrations of six polycyclic musks in blood samples from healthy volunteers.

Blood was taken from hundred healthy students of the Medical University of Vienna. The lipophilic fraction was extracted and after purification analyzed by GC–MS. Study participants also completed a questionnaire on the use of cosmetics, about nutrition and other life-style aspects.

Two compounds—galaxolide and tonalide—were identified in higher percentages of the blood plasma samples. Maximum plasma levels over 100 ng/l were also only found for galaxolide (4100 ng/l) and tonalide (800 ng/l). Women showed significantly higher levels than men. In a statistical multivariate approach only use of body lotion and age were predictive of positive galaxolide concentrations. For tonalide no significant predictor could be found.

The findings mirror the replacement of nitro musk fragrances by polycyclic musks, mainly galaxolide. The high concentrations of galaxolide in human blood raise concern since few toxicological data are available.

Keywords: Polycyclic musks; Galaxolide; Tonalide; Celestolide; Phantolide; Cashmeran; Traesolide; Human blood

1. Introduction

Synthetic musks are used as fragrances in numerous products, such as perfumes, soaps, body lotions, cosmetics, fabric softeners, laundry detergents, air fresheners, food additives and fish bait (Rimkus, 1999; Schmeiser et al., 2001). The first synthetic musks (dinitro- and trinitrobenzene derivates, so called nitro musks) were introduced into the market in the beginning of the last century (Rimkus, 1999). Since the 1950s polycyclic musk fragrances have also been used. Today, the production of polycyclic musk compounds is increasing, with a corresponding decline in nitro musk production (Rimkus, 1999; Peck and Hornbuckle, 2004).

* Corresponding author. Tel.: +43 1 4277 64727; fax: +43 1 4277 9647.

E-mail address: hans-peter.hutter@univie.ac.at (H.-P. Hutter).

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Both groups of chemicals are lipophilic and persistent in fat tissue. They bioaccumulate in the aquatic environment (Yamagishi et al., 1981; Eschke et al., 1994; Rimkus and Wolf, 1995; Fromme et al., 1999; Rimkus, 1999; Gatermann et al., 2002) and can be found in human fat tissue and breast milk (Liebl and Ehrenstorfer, 1993; Rimkus et al., 1994; Eschke et al., 1995; Müller et al., 1996; Rimkus and Wolf, 1996; Käfferlein et al., 1998; Liebl et al., 2000). In contrast to nitro musks, data on the toxicological properties of polycyclic musk compounds are sparse (Mersch-Sundermann et al., 1998; Liebl et al., 2000). In recent years, concerns have been raised about estrogenic activity of polycyclic musks (Bitsch et al., 2002; Schreurs et al., 2002).

Only few studies on synthetic polycyclic musks in human fat tissue and breast milk have been published (Eschke et al., 1995; Müller et al., 1996; Rimkus and Wolf, 1996; Liebl et al., 2000). Data on the concentration of polycyclic musk fragrance compounds in human blood are lacking. To our knowledge, only one study has been published so far (Bauer and Frössl, 1999).

The aim of this study was to determine the levels of polycyclic musks in blood plasma of young adults in Austria and to investigate the influence of gender, body fat, eating habits, use of cosmetics, etc. on the concentrations of these compounds.

2. Materials and methods

Blood samples were collected with Vacuette® plasma tubes (coated with sodium heparin) from 114 healthy students who attended courses at the Hygiene Institute of the Medical University of Vienna. Blood samples were put immediately after collection into a refrigerator at −20 °C. From these samples 11 were used for validation purposes of the assays, 100 samples served as basis for the analysis.

Height, weight, and subscapular skinfold thickness of the study participants were measured. Furthermore, subjects were asked if they had normal, dry or oily skin, how often they used body care products, how often they ate fish, etc.

2.1. Analytical procedure

2.1.1. Preparatory operations

Special care and several preparatory steps are necessary to avoid or reduce contamination of laboratory equipment with the musk compound analyts. Potential sources of contamination with musk compounds are laundry detergents, towels, cosmetics used by the staff, household cleaning agents applied in the laboratory but also the medical tubes used for taking blood samples. Different kinds of medical tubes were tested to select those with the lowest impact on blind values.

To remove potential residual contamination with musk compounds all glassware was dried at 200 °C for 12 h. In order to avoid losses by adsorption at the glass walls, the walls were rinsed with *iso*-octane before use. After the solvent was discarded, the residue solvent was evaporated under a fume hood. Glassware of the rotary evaporator was also rinsed prior to use. Sample preparation was carried out in a laminar flow chemical hood with a charcoal filter.

2.1.2. Sample preparation

The samples (8.5–9 ml) were defrosted and the internal standard (50 µl of a 100 pg/µl solution of deuterated tonalide-D3 in acetone) was added. Precipitation of the proteins was performed using acetonitrile (8 ml) and shaking vigorously. 3 ml *n*-pentane was added and shaking was continued for 2 min. The emerging coagulate was homogenised with a spatula. Extraction was carried out on a shaking board for 15 min followed by centrifugation (5 min: 2500*g*). The pentane phase was withdrawn to a new test tube. The extraction was repeated with *n*-pentane (2 ml); extraction time was 15 min (shaking board), centrifugation followed for 5 min at 2500*g*. This procedure was repeated twice. The combined pentane phases were concentrated and reconstructed to 1 ml hexane. Clean up was carried out with silica gel, conditioned by *iso*-octane, dichloromethane and *n*-hexane, each 5 ml. Elution was performed with hexane/ethyl acetate (4:1) (8 ml), dichloromethane (8 ml) and ethyl acetate (10 ml). The elute was collected and concentrated (1 ml). Another clean up step included elution on an aluminium oxide column (coated with sodium sulphate: 0.5 g) with hexane as solvent (10 ml). Therefore 2 g aluminium oxide were treated overnight at 450 °C and deactivated with 10% water. Prior to GC–MS analysis hexachlorobenzene (HCB) $^{13}C_6$ as injection standard was added.

With each sample batch, a blank sample was analyzed. For blank samples 8 ml of acetonitrile were spiked with 50 µl of a 100 pg/µl solution of tonalide-D3 in Acetone. The rest of the sample preparation followed the method described above. Average ($n = 11$) blind values range from 30 ng/l (tonalide) up to 50 ng/l (galaxolide). For celestolide, phantolide, cashmeran, traesolide blind values are below LOD (Limit of Detection).

2.1.3. Analysis

The GC–MS analysis was carried out by a Thermoquest Trace GC/MS System with negative chemical ionisation using selected ion monitoring (SIM) mode (Thermo Electron, USA). Ammonia was applied as reagent gas. A fused silica capillary column (60 m × 0.25 mm i.d., 1.4 µm film thickness) of crosslinked DB-624 (J&W, USA) was used. The operating conditions were as follows: column temperature, programmed from 110 °C to 210 °C at 15 °C/min, from 210 °C to 270 °C at 6 °C/min, and hold at 270 °C for 18 min. Injection tem-