ELSEVIER

Chemosphere 61 (2005) 422–431

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Synthetic musk fragrances in trout from Danish fish farms and human milk

Lene Duedahl-Olesen [a,*], Tommy Cederberg [a], Karin Høgsbro Pedersen [b], Arne Højgård [c]

[a] *Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*
[b] *Regional Veterinary and Food Control Authority Copenhagen, Flæsketorvet, DK-1711 Copenhagen, Denmark*
[c] *Regional Veterinary and Food Control Authority Århus, Sønderskovvej 5, DK-8520 Lystrup, Denmark*

Received 11 December 2003; received in revised form 10 January 2005; accepted 2 February 2005
Available online 13 March 2005

Abstract

Synthetic musk compounds used in detergents and cosmetics include nitro and polycyclic musk compounds. These compounds are discharged after use via domestic wastewater and sewage treatment plants to the aquatic environment. Quantitative detection of nitro musk and polycyclic musk compounds by GC/HRMS in Danish farmed trout and human milk from primiparous mothers are reported. The polycyclic musk, HHCB, dominated the synthetic musk compounds found in trout samples from 1999 with a median concentration of 5.0 μg/kg fresh weight (n.d.–52.6 μg/kg fresh weight) and in trout samples collected in 2003 and 2004 with a median concentration of 1.2 μg/kg fresh weight (n.d.–28.0 μg/kg fresh weight). It was also found that the concentration of musk xylene in trout sampled at the same fish farms decreased considerably from a median concentration of 5.1 μg/kg fresh weight in 1992 to a median of 0.5 μg/kg fresh weight in 1999 and to a median less than the detection limit (0.23 μg/kg fresh weight) in 2003. HHCB also dominated in Danish human milk samples collected in 1999 with a median concentration of 147 μg/kg fat (38.0–422 μg/kg fat). Human dietary intake assessment and body burden calculations on data from 1999 indicate that the main source of exposure to human cannot be attributed to the consumption of farmed trout.

Keywords: GC/MS; Musk xylene; Musk ketone; HHCB; Body burden

1. Introduction

Synthetic musk compounds are used in most fragrances for detergents, fabric softeners, cleaning agents, and cosmetic products such as soaps, shampoo and perfumes. Synthetic musks include nitro musks and polycyclic musks. For the two most widely used nitro musks, musk xylene (1-*tert*-butyl-3,5-dimethyl-2,4,6-trinitrobenzene) is primarily used in detergents and soaps and musk ketone (4-*tert*-butyl-2,6-dimethyl-3,5-dinitroacetophenone) is principally used in cosmetics (Sommer, 1993; Struppe et al., 1997). Musk ambrette (6-*tert*-butyl-3-methyl-2,4-dinitroanisole), musk tibetene (1-*tert*-butyl-3,4,5-trimethyl-2,6-dinitrobenzene), and musk moskene (1,1,3,3,5-pentamethyl-4,6-dinitroindane) are nitro musks used for detergents in smaller amounts. Polycyclic

* Corresponding author. Fax: +45 72 34 74 48.
E-mail address: ldom@dfvf.dk (L. Duedahl-Olesen).

0045-6535/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2005.02.004

musks are mainly used in detergents and include 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta(γ)-2-benzopyran abbreviated as HHCB (e.g. Galaxolide®), 6-acetyl-1,1,2,4,4,7-hexamethyl-tetralin abbreviated as AHTN (e.g. Tonalide®), 4-acetyl-6-*tert*-butyl-1,1-dimethylindane, abbreviated as ADBI (e.g. Celestolide®), 6-acetyl-1,1,2,3,3,5-hexamethylindane, abbreviated as AHMI (e.g. Phantolide®), 6,7-dihydro-1,1,2,3,3-pentamethyl-4-(5H)-indanon abbreviated as DPMI (e.g. Cashmeran®), and 5-acetyl-1,1,2,6-tetramethyl-3-isopropyl-dihydroindane abbreviated as ATII (e.g. Traseolide®). Musk xylene, musk ketone, HHCB and AHTN represent about 95% of the market in Europe whereof approximately 74% is HHCB (OSPAR Commission, 2000).

Synthetic musk compounds, initially musk xylene and musk ketone, were detected and identified for the first time in 1981 in freshwater fish from the Tama River in Tokyo (Yamagishi et al., 1981). A few years later, analysis of fish muscle, fish viscera, mussels, water and wastewater from the same river (Yamagishi et al., 1983) indicated that musk xylene and musk ketone accumulate in lipophilic tissues in biota. Ten years later, nitro musks were identified in the aquatic environment and lipophillic tissues, when Rimkus and Wolf (1993b, 1995) analysed fish, mussels and shrimps from various locations and started a broad discussion in the field. At the same time other authors published data on polycyclic musk levels in fish, surface and sewage water (Eschke et al., 1994a, 1995b). Nitro musks and polycyclic musks in humans have been detected by analysis of human adipose tissue, breast milk and human serum (Liebl and Ehrenstorfer, 1993; Rimkus et al., 1994; Rimkus and Wolf, 1995; Eschke et al., 1995a; Ott et al., 1999; Eisenhardt et al., 2001). These and more recent data (Draisci et al., 1998; Rimkus, 1999; Gatermann et al., 1999, 2002a) confirmed the presence of synthetic musk fragrances in the aquatic environment and the ubiquitous distribution of polycyclic musks at higher levels than for the nitro musk compounds. Synthetic musk compounds can be absorbed in human lipophilic tissues by dermal contact, however, recent studies based on the excretion in urine and faeces over five days indicate that this absorption might be less than 4% of dermal applied musk ketone, musk xylene or musk ambrette (Hawkins et al., 2002).

Due to neurotoxicity on rats (Ford et al., 1990) and potential photosensitivity in humans (Cronin, 1984; Parker et al., 1986) the use of musk ambrette was phased out and finally banned in 1995 (European Commission, 1995). Male rats have been found to develop testicular atrophy after administration of musk xylene and musk ketone (Ford et al., 1990) and the compounds are known to have a DNA damaging effect, a reproductive toxicity (Eisenhardt et al., 2001) and to function as co-mutagenic substances for polycyclic aromatic compounds (Mersch-Sundermann et al., 2001). Due to the bioaccumulation potential and toxicological impact of musk xylene, it was included in the "third list of chemicals for priority action" of the EU in 1997 (European Commission, 1997). Today, the use of musk tibetene and musk moskene are banned for cosmetics and personal care products (European Commission, 1998), whereas the concentration of musk xylene and musk ketone for use in the same products are regulated and are to be phased out (European Commission, 2002). The polycyclic musks, HHCB and AHTN are due to the use as high-volume chemicals included in the "fourth list of chemicals for priority action" (Gatermann et al., 2002a).

German analysis of trout from foreign fish farms (mainly Danish farms) collected in 1990 and 1992 showed increased levels of musk xylene and musk ketone (Rimkus and Wolf, 1993b). It was speculated that fish feed could be the source of contamination of musk xylene, but analysis of fish feed ($n = 175$) used in Denmark in 1992 showed that fish feed only contained a low average concentration of musk xylene of 1.2 μg/kg (Green, 1994). The source of the musk compounds was then expected to be from environmental pollution.

Surveillance on synthetic musk compounds has mainly included data on fish, human milk and adipose tissues from Germany. This study presents data on the levels of five nitro and five polycyclic musk compounds in trout from Danish fish farms sampled in 1999, 2003 and 2004. Comparison of levels of bromocyclene and musk xylene in Danish farmed trout from 1999 with levels in trouts sampled from the same fish farms as analysed in 1992 as well as musk xylene data on trouts collected in 2003 are included. Due to the lipophilic nature of the synthetic musk compounds and the possibility of exposure and accumulation in humans 10 human milk samples collected in 1999 from Danish primiparous mothers were analysed as well.

2. Materials and methods

2.1. Trout sampling and fat extraction

Three to five trouts were collected from the same pond at 50 different fish farms in the western part of Denmark. The collected fishes were wrapped and frozen separately. Sampling was done at Danish fish farms in the periods from January to March 1992 and repeated from September 1999 to January 2000 and from September 2003 to January 2004. Three to five trouts were collect from 35 suppliers from April to June 2004 at fish factories in Denmark.

For fish sampled in 1992 at least three fish were filleted and blended to a homogenate with a Moulinex food processor, whereas in 1999 and 2003/2004 five fish were filleted and blended. In both preparations guts and fins were removed from the fish prior to homogenisation.