POLYCYCLIC MUSK COMPOUNDS IN HIGHER TROPHIC LEVEL AQUATIC ORGANISMS AND HUMANS FROM THE UNITED STATES

Kurunthachalam Kannan, Jessica Reiner, Sehun Yun, Emily Perrotta, Lin Tao, Boris Johnson, Bruce Rodan

Introduction

Polycyclic musk compounds such as, HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta [g]-2-benzopyran; trade names include Galaxolide®) and AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene; trade names include Tonalide®) are used as fragrances in a variety of consumer products, including washing and cleaning agents and personal-care products (e.g., perfumes, aftershave lotions, body lotions, shampoos). In the United States, HHCB is listed by the U.S. Environmental Protection Agency (EPA), as a high production-volume chemical, which suggests that the production is more than 4500 tons per year, for uses that are reportable under the Toxic Substances Control Act. Due to their property of lipophilicity (log K_{ow} values of 5.4-6.3), HHCB and AHTN have been shown to accumulate in several aquatic organisms, such as fish and mussels [1-3]. Similarly, occurrence of HHCB and AHTN in human adipose tissues from Germany and Switzerland has been reported [4,5]. Recently, HHCB and AHTN have been shown to accumulate in the tissues of finless porpoise and shark collected from Japanese coastal waters [6]. Nevertheless, only a few studies have been conducted on the environmental distribution of synthetic musks in the United States [7,8]. In this study, we have measured concentrations of HHCB and AHTN in a variety of higher trophic level aquatic organisms and human tissues collected from the United States. To our knowledge, this is the first study to document the occurrence of these compounds in biological matrices from the United States.

Materials and Methods

Human adipose fat samples were obtained from a hospital in New York City during 2003-2004 (Table 1). Livers from polar bears (*Ursus maritimus*), originating from coastal waters of Alaska, were collected from native subsistence hunters. Livers from sea otters (*Enhydra lutris nereis*), harbor seals (*Phoca vitulina*), and California sea lions (*Zalophus californianus*) were acquired from the Marine Mammal Center, Sausalito, California; these animals had been found stranded along the central California coast. Blubber from bottlenose dolphins (*Tursiops truncatus*), spinner dolphins (*Stenella clymene*), and pygmy sperm whales (*Kogia breviceps*) were collected from animals stranded in Florida coastal waters. Livers from Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) were collected from Florida coastal waters. Livers from river otters (*Lutra canadensis*) and mink (*Mustela vison*) were from Michigan and Illinois, respectively. Common merganser (*Mergus merganser*), lesser scaup (*Aythya affinis*), greater scaup (*Aythya marila*) and mallard (*Anas platyrhynchos*) were collected from Buffalo, New York. Atlantic salmon (*Salmo salar*) were collected between August 2003 and March 2004 from retail markets in New York. Smallmouth bass (*Micropterus dolomieu*) were collected from Rock Pond and Effley water reservoir in New York in 2003.

The method used in this study was similar to those reported earlier [3,4], with some modifications. Briefly, sample tissues (1-5 g) were Soxlet extracted and spiked with 75 ng of d_{10}-phenanthrene. Lipid in the sample extract was removed by gel permeation chromatography (GPC) using a Bio-beads S-X3. The extract was then passed through a cartridge packed with 0.5 g of silica gel (100-200 mesh; Aldrich, Milwaukee, WI, USA) for cleanup. The solvent was concentrated to 200 mL or 1 mL, and was then injected into a gas chromatograph interfaced with a mass spectrometer (GC-MS, Agilent Technologies 6890 GC and 5973 Series MS). The ions were monitored at m/z 243, 258, 213 for HHCB; 243, 258 and 159 for AHTN; and 188 for d_{10}-phenanthrene. The GC column used was a DB-5 (5%-Phenyl-methylpolysiloxane, Agilent Technologies, Foster City, CA, USA) fused silica capillary column (30 m x 0.25 mm i.d.).

Results and Discussion

HHCB was found in all of the human fat samples analyzed, at concentrations ranging from 6.1 to 435 (mean: 97) ng/g, on a wet weight basis. AHTN was found in 86% of the samples analyzed, at concentrations ranging from <5 to 64

(mean: 23) ng/g, wet weight (Table 1). The overall mean concentration of AHTN in human fat was four-fold lower than the mean HHCB concentration. On a lipid weight basis, concentrations of HHCB and AHTN measured in our study were in the ranges of 12 - 798 (mean: 178) and <8-134 (mean: 42) ng/g, lipid weight, respectively; these values are two- to three-fold higher than those measured in German and Swiss adipose fat samples collected 10 years ago [4,5]. HHCB and AHTN have been reported in human fat samples collected from Germany during 1993-1995 at concentrations ranging from 28 to 189 (mean : 82) ng/g lipid weight for HHCB, and from 8 to 33 (mean: 19) ng/g lipid weight for AHTN.

Table 1. Concentrations of HHCB and AHTN (ng/g) in human adipose fat from New York City, USA

		HHCB	AHTN	HHCB	AHTN
	Fat %	Wet weight		Lipid weight	
Male (n=12)					
Mean±SD	34±9	72.2±75	20.5±14	136±143	39±29
Median	33	49.3	17.0	90.5	31.5
Range	21-51	6.1-251	<5-54	12-509	<8-110
Female (n=37)					
Mean±SD	31±7.3	105±90	23.5±11	192±170	43±22
Median	31	84.6	21.3	180	38.7
Range	18-51	10.6-435	<5-64	18-798	<8-134
Overall (n=49)					
Mean±SD	32±7.8	96.9±88	22.8±12	178±166	42±24
Median	32	73.7	19.8	149	37.4
Range	18-51	6.1-435	<5-64	12-798	<8-134
% Positive		100	86	100	86

Values below the quantitation limit were not included in the calculation of mean.

There was no significant relationship in the concentrations of HHCB and AHTN with age- or gender. The lack of age- or gender-dependent increase in the concentrations, coupled with elevated concentrations in individuals aged 25-35 years, supports the hypothesis that dermal application is a source of exposure to humans. Nevertheless, inhalation related exposure cannot be ruled out. Specifically, individuals in the age range of 25-35 years are expected to apply more musk-products. Further studies are needed to examine the sources of exposure. Lack of age-dependent increase in concentrations of HHCB and AHTN suggests metabolism and excretion of these compounds. If metabolism were slow in humans, then there would be an age dependency, as continued use of musk products would lead to buildup in the body. Because there is no age dependency, the data does suggest metabolism. HHCB has been shown to be transformed to HHCB-lactone in fish. Concentrations of HHCB in human fat were significantly correlated with AHTN concentrations ($p<0.01$), which suggests co-exposure of humans to the two musks.

HHCB and AHTN were found in tissue samples from marine mammals, birds, and fish (Figure 1). Among the wildlife species analyzed, the highest concentration (on a wet weight basis) was found in the blubber of dolphins collected in coastal Florida. The measured concentrations of HHCB in dolphins were within the range of concentrations reported for finless porpoises (*Neophocaena phocaenoides*) from Japanese coastal waters[6].

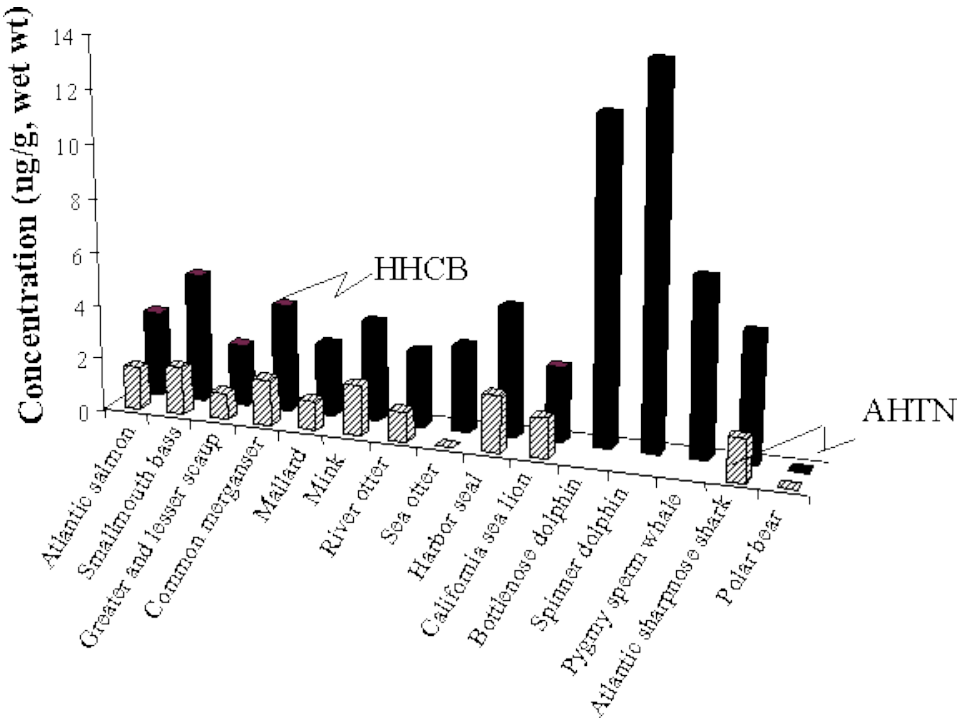

Fig. 1. Concentrations of HHCB and AHTN in wildlife tissues (liver or muscle) from the USA.

When the concentrations in our study were normalized on a lipid basis, the highest HHCB concentration, 183 ng/g, was found in the blubber of an adult male spinner dolphin. Concentrations of HHCB in bottlenose dolphin were in the range of 12-76 ng/g, lipid weight, whereas the lowest concentration was found in pygmy sperm whale (7.4 ng/g, lipid weight). HHCB was detected in liver tissues of seals, sea lions, Atlantic sharpnose shark, river otter, mink, common merganser, lesser scaup, greater scaup, and mallard. Concentrations of HHCB in the livers of these wildlife species were between 1.5 and 5.3 ng/g, wet weight. Fish species such as Atlantic salmon and smallmouth bass also contained detectable concentrations of HHCB.

Discharges from wastewater treatment plants have been suggested as an important source of HHCB and AHTN for surface waters, and thereby for aquatic organisms. Several studies have reported the occurrence of HHCB and AHTN in sewage treatment plant influent and effluent samples.

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