DETERMINATION OF SYNTHETIC MUSK COMPOUND LEVELS IN INDOOR AIR

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ABSTRACT

DETERMINATION OF SYNTHETIC MUSK COMPOUND LEVELS IN INDOOR AIR

Synthetic musk compounds are one group of semivolatile organic compounds (SVOCs), and they are generally used as ingredients for odor in products such as detergent industry and cosmetics. Nowadays the increase in the use of synthetic musks caused increased in the production rate of these compounds. As a result they are detected in air, water, and aquatic biota. Like other SVOCs, they are classified as bioaccumulative, toxic and endocrine disrupting chemicals.

In this study, 10 indoor air gas and particulate phase samples were collected from primary school classroom and woman sport center due to sensitivity of the group of people: children and woman in high activity. Particulate phase and gas phase synthetic musk compounds concentrations were determined. The synthetic musk compounds studied in this research were Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI), Traseolide (ATII), Phantolide (AHMI), Cashmeran (DPMI), Musk Ketone (MK), Musk Xylene (MX).

The analyzed samples were showed the gas phase and particulate phase concentrations in primary school classroom were higher than sports center. All synthetic musk compounds were found in the gas phase samples in the primary school. Except musk ketone, the rest of the compounds were detected in the sports center. The gas phase concentration for classroom ranged from 267±56 (HHCB) to 0.12±0.2 ng /m^3 (MK) while it varied from 144±60.6 (HHCB) to 0.08±0.1 ng/m^3 (AHMI) for Sports Center. The order of the compounds in the samples for the sampling places showed differences. From the highest to lowest concentration order was HHCB>AHTN>ATII>DPMI>MX>ADBI>AHMI and HHCB> DPMI >AHTN>ATII> >MX>ADBI>AHMI>MK for sports center and classroom respectively.

ÖZET

SENTETİK KOKU BİLEŞİĞİ SEVİYELERİNİN İÇ HAVADA SAPTANMASI

Yarı uçucu organik bileşiklerden olan sentetik koku bileşikleri banyo ve kozmetik gibi endüstriyel ürünlerde kullanılmaktadır. Günümüzde sentetik kokuların kullanımının artması üretimlerinin de artmasına sebep olmaktadır. Bunun sonucu olarak havada, suda ve suda yaşayan canlılarda tespit edilmektedir. Diğer yarı uçucu organik bileşikler gibi canlı dokularında biriken zehirleyici ve endokrin bozucu kimyasallardır.

Bu çalışmada, on tane gaz ve partikül fazında iç hava örnekleri çocuk ve bayanların yüksek aktivitelerine göre ilkokul sınıfı ve bayan spor salonundan alınmıştır. Partikül madde yoğunluğu, partikül faz ve gaz fazındaki sentetik koku maddelerinin konsantrasyonları ölçülmüştür. Bu çalışmada Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI), Traseolide (ATII), Phantolide (AHMI), Cashmeran (DPMI), Musk Ketone (MK), Musk Xylene (MX) sentetik koku bileşikleri araştırılmıştır.

İncelenen örneklerde sınıf örneklerinde bulunan gaz fazı ve partikül fazı konsantrasyonları spor merkezi örneklerinden daha yüksek bulunmuştur. İlkokul örneklerinde bütün sentetik koku bileşikleri bulunmuştur. Spor merkezi örneklerinde ise musk ketone haricinde bütün sentetik kokular bulunmuştur. İlkokul örneklerinde gaz fazı konsantrasyonları 267±56 (HHCB) ile 0.12±0.2 ng/m^3 (MK) arasında, ilkokul örneklerinde 144±60.6 (HHCB) ile 0.08±0.1 ng/m^3 (AHMI) arasında değişmektedir. Örneklerde bulunan bileşik değerlerinin düzeni değişmektedir. İlkokul örneklerinin konsantrasyon değerlerinin yüksekten aza doğru sıralaması HHCB> AHTN> ATII> DPMI> MX> ADBI> AHMI sırasında olup spor merkezi için HHCB> DPMI >AHTN>ATII> >MX>ADBI>AHMI>MK sırasındadır.

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CHAPTER 1

INTRODUCTION

Anthropogenic organic chemicals are the big concern of environments due to their environmental resistance to degradation and the ability of mimicking like natural hormones, therefore they create disturbances on the functions of body. These kinds of chemicals are increasing in the environment. Many of them are in the category of persistent organic chemicals. These are able to bind estrogen receptor and influence estrogen signaling pathway therefore most of them are called as “endocrine disruptor”.

Natural musks are originally were extracted from musk ox and musk deer‘s (*Moschus moschiferus*) exocrine gland secretions which is situated between its stomach and genitals (Fromme et al., 2003). These substances one of the most expensive animal products in the world have been used as a perfume fixative since ancient times. The musk deer, which lives largest number of in China and Russia, and also Pakistan, India, Tibet, Siberia and Mongolia, belongs to the family of *Moschidae*. To obtain the musks, the deer is killed and its gland, also called "musk pod", is removed. Musks also can be obtained some plants such as *Angelica archangelica* or *Abelmoschus moschatus* that produce musky smelling macrocyclic lactone compounds. These compounds are also widely used in perfumery as substitutes for animal musk or to alter the smell of a mixture of other musks (Gebauer and Bouter, 1997). Due to the difficulty and uncertainity of natural supply, they are costly; therefore, this group of chemicals has been replaced by synthetic musks with odoriferous characteristics similar to natural compounds (Peck et al., 2006). These synthetic musks are a heterogeneous group of chemicals and no similar structural or chemical relationship with natural ones.

In the beginning of 20th century tri-nitro and di-nitro benzenes, the groups of the so-called nitro musks were used as the first synthetic musk fragrances. Musk Xylene (MX) and musk ketone (MK) have been used widely in the many consumer products as ingredients in perfumes, cosmetics soaps, laundry detergents, fabric softeners and household cleaners. In the 50’s the other groups with non-nitro benzenoid structure called polycyclic musks were introduced to the market which have been used to the all consumer products similar where nitro musks used as ingredients. In 1996 the worldwide production of synthetic musks was about 8000 tons/year while 70 % of this total world market was shared by the polycyclic musks species Galaxolide and Tonalide and the rest mainly by Musk xylene and Musk ketone. Nowadays a general increase in the polycyclic musk production and corresponding decline in the nitro musk were observed (Gebauer and Bouter, 1997).

Synthetic musks have natural musk-like odour and have a positive effect on the quality of a fragrance. Because of their ability to bind fragrances to fabrics and to the skin, synthetic musks make fragrances more balanced and longer lasting. Synthetic musks properties relate to their low volatility and water solubility, and high solubility in organic solvents and tissues.

The major source of synthetic musk compounds in the environment through the wastewater discharge to aquatic environment. Therefore the study in the area focused on the analysis of these compounds in wastewater, sediment samples and aquatic animals such as fish, mussel etc. The first determination of synthetic musks in environmental compartments was observed in 1980's (Kallernborn et al., 1999). Considerable concentrations of musk xylene and musk ketone were found in biota and water samples in Japan. These first results showed the resistance to degradation of synthetic musks due to their lipophilic nature. In 1990's nitro musks were analyzed in fish and mussels in different locations and first time they were found in the human milk and adipose tissue. Later, Germans detected polycyclic musk fragrance in water, fish and some municipal sewage treatment plants (Rimkus et al., 1999).

The European Union limited the use of musk ketone and musk xylene around 0.03-1% and 0.042-1 % respectively due to being possible carcinogens and induction of metabolizing enzyme. They also recommended cautious use for the consumer products containing 4% musk ketone (SCCNFP, 2004).

The evidences about the distributions of the musks in environmental media, aquatic food chain, as well as adipose tissue and mother's milk brought the attention on children and elders with weakened immune systems due to particular susceptibility to synthetic musks. Each and every system of the body may be adversely affected by synthetic musks (Wolff, 2005). According to the Environmental Protection Agency, like semi-volatile organic compounds (SVOCs), synthetic musk compounds affect the health. Asthma, reactive airway disease (RADS), difficulty in breathing, upper respiratory problems, skin problems immune system damages (Mass. Nurses Ass., 2009) can be seen. Additionally, cosmetics and fragranced products can also pose high risks for breast cancer and other illnesses. So, people who have been already have these health problems and chemically injured are particularly vulnerable (Wolff, 2005). The Institute of Medicine placed fragrances in the same category as second hand smoke in triggering asthma in adults and school age children (Natural Ingredients, 2009). The study shows that the 72% of asthmatics had negative reactions to perfumes. The most common issue for fragrances is the skin allergies and they might be caused dermatitis, itchy or burning skin (Wolff, 2005). Synthetic musks are lipophilic compounds as a result sorption from cosmetics and detergents caused a dermal contamination route. Allergic reactions can be caused of possibility for hormonal effects by biocides in the fragrances. Moreover these compounds inhibit the activity of multidrug efflux transporters responsible for multixenobiotic resistance (MXR) in gills of the marine mussel (Luckenbah et al., 2005).

However, fragrances might be a problem for the indoor climate. During the cleaning process or after cleaning the user of the cleaned places will be exposed to the fragrances and if people are sensitive, fragrances might cause headache and respiratory irritation. Only few molecules are enough to induce such reactions. Special attentions should be taken for products that might cause problems for these groups. Not only soaps, shampoos, lotions and other products in direct dermal contact are well known as a problem for these peoples, but even clothes washed with detergents or using deodorants, perfume and other contact by smelling may cause severe problems (Swedish Soc. Nat. Cons. Foun., 2009).

When it comes to study related to air concentration a few study are available in the literature. The exposure to these kinds of compounds can be through dermal, ingestion and inhalation pathways. High exposure through inhalation pathway is possible in the use of these compounds for cleaning or personal products regularly. In this study polycyclic and nitro musk compounds will be determined in two different indoor environment air. One is the classroom in the primary school, while the other is the sports center where only women are the members. Primary school kids' age ranges from 7 to 14 who are in the growth age and spend their school time mostly in the classroom. The cleaning schedule of the classrooms is din daily routine with household commercial cleaners while in the sports center is also cleaned daily with a natural soap product called "yellow soap". The sports center is a place where the members have high activity in a short time. They are also common user of personal care products. The women can get affected by synthetic fragrances through personal care products and cleaning agents combined with high breathing rate. Both children and women are the potential effected people to synthetic musk fragrances.

In this study the concentration levels of these compounds in the gas and particulate phase will be determined. In the second chapter of thesis the studies related to synthetic musk compounds will be gathered and classified from literature. In the chapter three the sampling, preparation and analysis methods will be summarized. The results and discussion part will be found in 4th chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Properties of Synthetic Musk Compounds

Synthetic musks are used as an alternative for the natural musk and comprise a broad variety of structurally heterogeneous compounds. They are structurally complex phototoxic, chemically lipophilic, resistant and bioaccumulative chemicals. Table 2.1 shows the chemical and physical selected properties of these compounds. Mainly they are divided into two major groups. The first group is polycyclic musks, which are acetylated and highly methylated pyran, tetralin, and indane skeletons, and their trade names are Tonalide, Galaxolide, Traseolide, Celestolide, Phantolide, and Cashmeran. The second group is called as nitro musks methylated, nitrated, and acetylated benzene ring and commonly used nitro musks trade names are Musk Xylene and Musk Ketone. Musk Xylene is produced as an inexpensive substitute for natural musk and musk ketone, and is a fixateur and very stable in cosmetics, soaps, and detergents (Swedish Soc. Nat. Cons. Foun., 2009).

Natural musks can be obtained from animal's exocrine gland secretions or extracted from some plants (Fromme et al., 2003). Good musk has a dark purplish color, dry, smooth and bitter in taste, also dissolves in boiling water; alcohol takes up, ether and chloroform dissolve still less. However, it contains ammonia, cholesterol, fatty matter, a bitter resinous substance, and other animal principles (Wikipedia, 2009). The plant originated musks produce musky smelling macrocylic lactone compounds. These compounds are widely used in perfumery instead of animal musks through, and alter the smell of a mixture of other musks (Gebauer and Bouter, 1997). Because of obtaining the deer musk requires killing the animal, nearly all musk fragrances used in perfumery today are synthetic, and called "white musk". Nowadays synthetic substances are industrially and commercially produced in large quantity. Synthetic musks have natural musk-like odour and have a positive effect on the quality of a fragrance. Synthetic musks are widely employed for cosmetics, like lipstick and perfumes, toiletry industry, many kinds of cleaning, polishing and washing agents, like toothpaste, shampoo, soap and household products, like cleaning spray and aromatic oils (Fromme et al, 2003). Some synthetic musk fragrances might have antimicrobial effect.

Increase in the use of synthetic musks is caused by increase in the production rate of these compounds. In 1988 global production of synthetic musks was 7000 tones, but this production rate has been increased since than. 110 tones musk xylene, 61 tones musk ketone, 1482 tones HHCB and 585 tones AHTN were used in only Europe in 1995 (Lutter, 2000). Today, the use polycyclic musks is common in Europe whereas the nitro musks usage is common in United States fragrance industries. Furthermore the worldwide leaders of products and exporters of musk fragrances are Chinese and Indian manufacturers (Kallernborn et al., 1999).

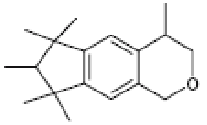
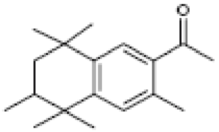
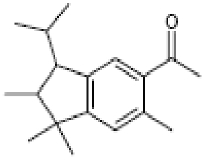
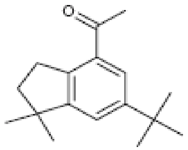
Currently MX, MK, AHTN and HHCB represent about 95 % of the market in Europe for all nitro musks and polycyclic musks. After 1992, the use of MX, MK and AHTN has been decreasing however the use of HHCB is increasing. Macrocyclic musks (ketone and lactone), on the other hand share the smallest market with a production level of 100 tons worldwide.

Due to the higher fragrance qualities and the concerns about potential toxicity the trend is to replace the nitrocyclic musks with macrocyclic and polycyclic musks. In the other words the production and use are changing from nitro musks to macrocyclic musks (Nitro musk → Polycyclic musk → Macrocyclic musk (Brassylic acid)).

Polycyclic musks are not biodegradable, thus more companies try to produce macrocyclic musks like Brassylic acid. Macrocyclic musk components are in contrast to nitrocyclic and polycyclic substances, have no health hazards and are biodegradable. The biodegradation tests show that four nitro musks (MX, MK, Musk Moskene (MM) and Musk Tibetene (MT)) do not mineralize under standard test conditions. However, macrocyclic musks were easily biodegradable and have a structure more similar to naturally occurring musks (Swedish Soc. Nat. Cons. Foun., 2009). Degradation data for some musk compounds are given in Table 2.2.

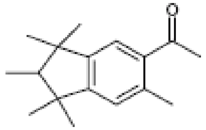
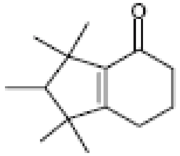
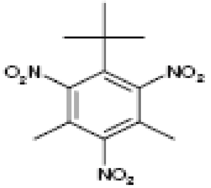
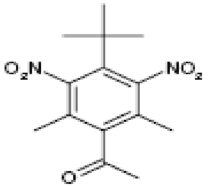
Due to detecting synthetic musks in environmental media, their increasing production rate and effects of health The European Scientific Committee on Cosmetics has accomplished that human exposure to musk should be reduced (Lutter, 2000).

Table 2.1. Synthetic Musk Fragrances and Selected Properties

Compound Name	**Trade Name**	**Mol Wt.**	**S(mg/L)**	**H (Pa m^3/mol)**	**log K_{ow}**	**Vapor Pressure (Pa)**	**Structure**
1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamrthylcyclopenta-(g)-2-benzopyran (HHCB)	Galaxolide	258.4	1.75	11.3	5.9	0.073	
1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone (AHTN)	Tonalide	258.4	1.25	12.5	5.7	0.068	
3-(1-methylethyl)-1H-inden-5-yl]-ethonone1-[(2R-3R)-2,3-dihydro-1,1,2,6-tetramethyl (ATII)	Traseolide	258.4	0.085	85.1	8.1	1.2	
1-[6-(1,1-dimethylethyl)-2,3-dihydro-1,1-dimethyl-1H-inden-4-yl]-ethanone (ADBI)	Celestolide	244.3	0.015	1801	6.6	0.020	

(cont. on next page)

Table 2.1. (cont.)

Compound Name	**Trade Name**	**Mol Wt.**	**S(mg/L)**	**H (Pa m^3/mol)**	**log K_{ow}**	**Vapor Pressure (Pa)**	**Structure**
1-[2,3,dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl]ethanone (AHMI)	Phantolide	244.3	0.027	646	6.7	0.024	
DPMI 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one	Cashmeran	206.3	0.17	9.9	4.9	5.2	
MX 1-(1,1-dimethylethyl)-3,5-dimethyl-2,4,6-trinitro-benzene	Musk Xylene	297.2	0.49	0.018	4.9	0.00003	
MK 1-[4-(1,1-dimethylethyl)-2,6-dimethyl-3,5-dinitrophenyl]-ethanone	Musk Ketone	294.3	1.9	0.0061	4.3	0.0097	

International Fragrances Association was suggested that the concentration level of a fragrance compound in toilet soap is 1.5 %, in a shampoo 0.5 % and in a detergent 0.15 %. The amount of fragrances and colorants in powder and liquid laundry detergent may be 1 percent by weight content (Swedish Soc. Nat. Cons. Foun., 2009).

Table 2.2. Degradation data for some musk compounds
(Source: Swedish Soc. Nat. Cons. Foun., 2009)

Type of Musk	**Degradation data**
Nitro musks	
Musk ketone	Not inherently biodegradable
Musk xylene	Not readily biodegredable
Musk tibetene	Not inherently biodegradable
Musk moskene	Not inherently biodegradable
Polycyclic musks	
HHCB	Not readily biodegradable
AHTN	Not inherently biodegradable (<1% biodegradation after 28 days) Low mineralisation
ADBI	Not inherently biodegradable (<1% biodegradation after 28 days)
AHMI	Low mineralisation

2.2. The Level of Synthetic Musk Compounds in Environmental Media

Kallernborn et al. (1999) reported that the first evidence of musk in biological samples (fish) and river water was proven by a Japanese research group at the river Tama nearby Tokyo in the early 1980. After founding considerable concentrations in the environmental samples, the new evidences were published for European environment. High accumulation potential in biological system was obtained by the calculation on lipophilic properties and stability. Nowadays, using analytical methods synthetic musk compounds can be identified and quantified in variety of samples

2.2.1. Water and Wastewater

The studies for the determination of synthetic musk compounds in water were carried out in river and sea water, sediment and sewage samples. The levels of different musk compounds ranged from 0.09 ng/L (HHCB in North Sea) (Bester et al., 1998) to 6000 ng/L HHCB and 5000 ng/L MK in Swedish sewage waste water (Paxeus, 1996).

Quednow et al. (2008) detected synthetic musk fragrances in 175 samples and most of the collected from September 2003 till April 2005 in a region in the south of Frankfurt, Germany. The area was defined presence of abundant groundwater reservoirs, and thus the region was one of the major source of drinking water for the Frankfurt city. Concurrently, the area was densely populated and industrialized area affected by human construction activity and continuous input of municipal and industrial wastewater. Due to strong anthropogenic impact and transformation, negative impacts on natural draining streams were reported. The region is drained by four river systems, Schwarzbach, Modau, Winkelbach, and Weschnitz, which all of them flow from east to west and are tributaries to the Rhine River. The water samples were taken from surface waters. The concentrations from these rivers were ranged from 5 to 678 ng/L for HHCB and from 3 to 299 ng/L for AHTN, respectively. The authors concluded that due to the detection of the highest concentration measured in the Schwarzbach River was a result of getting high amount of treated wastewater from a densely populated area. The lowest concentrations were detected in Winkelbach River where surroundings were populated 4 times lower than Schwarzbach River surroundings.

The other study in Germany was conducted by Rimkus et al. (1999). He and his coworkers analyzed five water samples from the downstream of river Elbe in Hamburg for detection of MX, MK, and their monoamino metabolites. One sample was collected Lauenburg, one sample was collected Brunsbüttel and three sample were collected Neumühlen that all stations are the downstream of a sewage plant of River Elbe. The water concentrations were between 0.5 to 9 ng/l. The highest concentrations were detected in Neumühlen samples for MX's monoamino metabolites (4-NH_2-MX), which was < 1 to 4 ng/l. However MX was also detectable in very low concentrations at the same samples. MK and its monoamino metabolites were below the detection limits or at very low concentrations (Rimkus et al., 1999).

Moreover the river water samples were analyzed for polycyclic musks. The highest concentrations found in river cannels in Berlin where the sewage treatment plant waters discharged to channels. The highest concentrations were found for HHCB and AHTN for all water samples. Even though, the sea waters concentrations were lower than the river water, they found out that when the year increased the concentration of musks in sea water increased too. In 1990, HHCB and AHTN concentrations were found 0.09–0.88 and 0.09–0.94 ng/l, while HHCB and AHTN concentrations were increased 0.15–4.8 and 0.08–2.6 ng/l, in 1995, (Rimkus et al., 1999). This results can be attributed either the increase in use of the products containing the musks or the increase in the population in that area.

Chen et al. (2007) determined the concentration and distribution of polycyclic musks, DPMI, ADBI, AHMI, AHTN, and HHCB, in a cosmetic plant. For this purpose, the samples were collected from a cosmetic plant in the HuangPu industrial park in GuangZhou during 15 to 22 November 2004. Two influent and effluent samples were collected from the wastewater treatment plant attaching to the cosmetic plant for every 2 hour. DPMI, ADBI, AHMI, AHTN, and HHCB were detected for all influent concentrations, but only AHMI was not detected in effluent concentrations in all samples. Highest concentrations were detected for HHCB and AHTN both influent and effluent concentrations. The average concentrations were 32.06 μg/l for HHCB and 5.41 μg/l for AHTN in effluent samples, and 549.68 μg/l for HHCB and 64.60 μg/l for AHTN in influent samples. Even though the highest concentrations were found for HHCB and AHTN in both influent and effluent, the concentrations were decreased after wastewater treatment. According to average removal efficiency 92.09%, 90.58%, 94.17%, 91.63% and 93.80% for DPMI, ADBI, HHCB and AHTN they concluded that the polycyclic musks removal efficiency was very high in wastewater treatment.

Benotti et al. (2009) searched pharmaceuticals and endocrine disrupting compounds in United States drinking water. They conducted the study in 19 drinking water treatment plants between the year of 2006-2007. They collected the samples from source water, finished drinking water, and distribution system (tap) water to analyze 51 compounds like atenolol, carbamazepine, naproxen, trimethoprim as pharmaceuticals, and HHCB, AHTN, ATII, musk ketone, atrazine, estrone as endocrine disrupting compounds. All of the drinking water plants had various combinations of coagulation, flocculation, sedimentation, filtration, primary and secondary disinfection. They reported that seven plants were using ozone as a primary disinfection and the rest of the plants disinfection were done by chlorine and chlorine dioxide. One of the plant was using groundwater while the remaining used surface water as a source of water. They detected mostly 11 compounds: atenonol, carbamazepine, gemfibrozil, naproxen, phenytoin, sulfamethoxazole which are pharmaceuticals, and atrazine, estrone, tris (2-chloroethyl) phosphate (TECP) which are endocrine disruptor compounds. Only HHCB was detected among the analyzed synthetic musk compounds in water samples. The median concentration for four source water samples was 3 ng/l while it was 31 ng/l for three finished water samples. None of the synthetic musk compounds were detected in tap water.

2.2.2 The Concentration of Synthetic Musk Compounds in Sewage and Sediment

Rimkus et al. (1999) collected seven sewage samples from two sewage plants for obtaining MX, MK and their monoamino metabolites. The plants were located in the northern Federal State of Germany in Hamburg and Scheswig-Holstein. Two samples were taken from sewage pond in Scheswig-Holstein. The highest concentrations found for musk xylene and musk ketone in Hamburg sewage plant’s influent samples. The reported concentrations were 150 and 550 ng/l for MX and MK, respectively. They found out monoamino metabolites in influent samples at very low concentrations, but they were increased in effluent samples. They concluded that the reason would be the steric effect of tert-butyl group. The main pathway for transformation for this transformation was explained with the formation of para-isomer metabolite and the importance of transformation pathway in sludge. The adsorption of musks to sludge, leads the transformation of the nitromusks in the treatment plant and resulting with the decrease in the concentration of the parent compounds and the increase in the amino derivatives (Swedish Soc. Nat. Cons. Foun., 2009). They also mentioned that the discharges from the sewage plant into water were an important source for both nitro musks and their amines. Similar to effluent concentrations, they found out the sewage pond concentrations were also lower than influent samples.

Rimkus et al. (1999) observed the polycyclic musks concentrations for sewage plant in Germany, Sweden and Netherlands. The concentrations of polycyclic musks were much detectable than nitro musks. The highest concentrations were determined for HHCB with a range 1000 to 6000 ng/l in Sweden effluent samples, and 800 to 4400 ng/l for AHTN in Germany, whereas the nitro musks were not detected or low concentrations the same samples.

The analysis of sediments for four samples from river Elbe in Teufelsbrück and Wedel-Schulau and 2 sewage ponds in Schleswing-Holstein showed that the highest concentrations were detected with 800 and 6300 ng/kg wet weight for musk xylene and musk ketone respectively (Rimkus et al., 1999).

The concentrations of sediment sample from sewage pond were detected higher than both the river sediment samples and river water samples concentrations. When all water samples were compared by researchers the highest concentrations were detected for sediment because of accumulation, whereas the lowest value was detected for sea water.

Chen et al. (2007) searched the concentration and distribution of polycyclic musks, DPMI, ADBI, AHMI, AHTN, and HHCB, in a cosmetic plant. HHCB and AHTN were major compounds both primary and secondary sludge in the cosmetic plant. The average primary sludge concentrations were 512.45 mg/kg and 58.61 mg/kg for HHCB and AHTN, respectively. The secondary sludge concentrations were 565.67 mg/kg and 95.24 mg/kg for HHCB and AHTN. The other synthetic musk compound, DPMI, concentrations was found higher both primary and secondary sludge, too.

Chen et al. (2007) compared the concentration results of wastewater and sludge, and observed that polycyclic musk concentrations were higher in sludge than water. However, the concentrations were higher in secondary sludge than primary sludge. These results indicated that the synthetic musks were easily adsorbed to the sludge. The authors indicated that the activated sludge may not only important mechanism to remove of polycyclic musks but also a potential environmental pollution source.

2.2.3. The Concentration of Synthetic Musk Compounds in Biota

All endocrine disrupting chemicals have tendency to accumulate in body fat of human and animals. The first study was conducted by EPA team in conjunction with Baylor University. They analyzed fillet and liver tissue of fish to obtain personal care product (PCPs) concentrations. The researchers collected 18-24 months old adult 6 fish at 5 different sampling locations. They found out HHCB and AHTN in filet tissue, while ADBI, MK and MX were not detected (EPA, 2008) at all.

Wan et al. (2007) also measured the amount of seven musk fragrances in liver, muscle, heart, gonad, stomach, intestines, adipose, gill, pancreas, kidney, gallbladder, and roe in 13 female Chinese sturgeons. They got similar results with EPA they detected only HHCB, AHTN, and MX mainly in liver, adipose tissue, and roe and the rest of the musks' concentrations were detected below the detection limit. Due to the accumulation in body fat, they compared lipid ratio in the study, the more lipid ratio resulted in the higher concentration of musks.

Nakata et al. (2005) were determined two of polycyclic musks; HHCB, AHTN, MK, MX and MA for the investigation in the bioaccumulation in a marine food chain. Samples were collected from tidal flat and shallow water areas of the Ariake Sea (Japan) through 2000 till 2005 for several tropic species like lugworm, clam, crustacean, fish, marine mammal, and bird. HHCB and AHTN were also detected frequently in all samples but nitro musks were not detected any of the organisms. The highest HHCB concentrations were detected in the liver of eagle ray (51 ng/g wet weight) and finless porpoise (26 ng/g wet weights) in shallow water organism. When they normalized the concentrations on a lipid weight basis, the tidal flat area organisms had higher concentrations than shallow water area organisms for accumulation of HHCB. HHCB concentrations were detected also highest in clams (258 ng/g lipid weight to 2730 ng/g lipid weight) whereas in mallard and black-headed gull was very low. On the other hand they did not found any correlation between bioaccumulation of synthetic musks and the tropic status of organisms while poly chlorinated biphynels gave positive correlation. The authors concluded that these results were obtained due to the fact that the metabolism of these compounds in organism caused the elimination of HHCB in higher tropic organism and short half-life of HHCB in river water than PCBs. They compared the results on the time basis for marine mammals during 1997 to 2005 in Japan and found out increase in HHCB concentrations.

HHCB and AHTN have been detected with higher concentrations in many samples from wastewater, surface water, sewage sludge, suspended matter, sediments and biota. The prevalent presence of these substances in the aquatic environment may be caused by the continuous input of these chemicals through sewage treatment plant effluent discharges into the aquatic environment and the low degradation rates.

2.2.4. The Concentration of Synthetic Musk Compounds in Human Milk

Like adipose tissue, synthetic musks are expected to accumulate in human breast milk because of their lipophilic nature. The major route of exposure might be dermal adsorption of personal care products.

Reiner et al. (2007) conducted a research by collecting 39 milk samples from different aged woman from Massachusetts. 31 women who had not previously nursed a child and 7 women who had nursed a child one or more were involved in the study. The results showed HHCB had the highest concentration both previously nursed and not nursed woman. The highest synthetic musk concentration was found for HHCB (227 ng/g lipid weight) in a 31year old woman who had not previously nursed a child. After HHCB, the second highest average concentration was found MK with 83.3 ng/g lipid weight, AHTN with 50.5 ng/g lipid weight, and musk xylene with 29.2 ng/g lipid weight for milk sample which were obtained not previously nursed woman. However, the highest concentration was detected for musk xylene with 39.7 ng/g lipid weight, AHTN with 29.9 ng/g lipid weight, and MK with 25 ng/g lipid weight for milk samples which obtained from woman who had previously nursed one or more. However, there was no correlation between age and concentrations of musk xylene, musk ketone, HHCB, and AHTN in their study.

Ueno and his coworkers (2009) intended to measure two polycyclic synthetic musk fragrances (HHCB and AHTN) in human breast milk in Japan. The human breast milk samples were collected from four primipara women and one multipara woman in

Japan from 2006 to 2008. Total 20 human breast milk samples were collected a month after childbirth and five samples were collected monthly from each woman. The women ages changed between 27 to 38 for primapara women, and multipara woman age was 28. The frequency of detection of HHCB was 60 %, while it was 30 % for AHTN in 20 samples. The authors compared HHCB in breast milk samples with PCBs in same breast milk samples and PCBs they reported that in all breast milk samples PCBs were detected while HHCB was one of the major synthetic chemicals in Japan.

2.2.5. The Concentration of Synthetic Musk Compounds in Air

2.2.5.1. Ambient Air

There are not many studies available on the measurement of the synthetic musks. The first study was conducted by Kallerborn et al. (1999). The study contained ambient and indoor air samples from the South Norway both. They detected 223 pg/m^3 for HHCB and 64 pg/m^3 for MX in the ambient air respectively. They found out AHTN, ATII and musk ketone were also in detectable concentrations in both indoor and ambient air.

Peck and Hornbuckle (2004) studied the synthetic musks at the shore of Lake Michigan, south of downtown Milwaukee and over Lake Michigan. They determined all musks in Milwaukee and Lake Michigan except DPMI. HHCB and AHTN were detected in higher concentrations both over and shore of lakes. HHCB concentrations were detected 4.1 and 1.1 ng/m^3 for Milwaukee and over Lake Michigan respectively. Nitro musks concentrations were significantly found lower than polycyclic musks. MX was detected 0.032 and 0.014 ng/m^3 Milwaukee and over Lake Michigan.

Peck and coworkers (2006) continued the collection of ambient air samples in different part of the country. They got 181 gas-phase samples with high volume air samples with 0.21 to 0.58 m^3/min flow rate for 7.5 to 12.3 hours sampling time in urban (Metro), suburban (Iowa City-IA-AMS), rural sites of Iowa (Hill) and Great Lake, over

Lake Erie and Lake Ontario. The highest musk concentrations were detected in urban site (Metro). They measured HHCB and AHTN concentrations higher than other polycyclic and nitro musks. Depending upon the sampling sites the median concentrations of synthetic musk fragrance were 0.80 ng/m^3 for HHCB and 0.33 ng/m^3 for AHTN for urban area while lake and suburban site concentrations were detected close to each other. As a result they concluded that even though the urban site concentrations were higher than suburban and rural site for the same musk compounds (HHCB, AHTN) these compounds were also detected in the all sites.

Peters et al (2008) determined a total of six polycyclic musks, DPMI, ADBI, AHMI, AHTN, HHCB, ATII, and five nitromusks, (MA, MX, MK, MM, MT) in precipitation samples in Netherlands. The results for these compounds showed 3 polycyclic musks (DPMI, ADBI and AHMI) and two nitro-musks (MX, MM) were not found at all samples. Only MK, MT was found at three locations with concentrations up to 10 ng/L. They determined the banned synthetic musk compound MA in 17 out of the 50 samples. HHCB was found in all samples with measured concentrations ranging from 2.3 to 25 ng /L. They concluded that HHCB concentrations were spread over all sample locations due to diffusive emissions from the source of consumer and domestic use. Moreover AHTN was found in 44 of the 50 samples and authors interpreted that emission was from the center of the Netherlands due to the presence of a production plant in the area.

2.2.5.2. Indoor Air

Due to common use of cleaning agents, other personal and household products determinations of the synthetic musks in indoor environment are more important for the exposure. Fromme and coworkers (2003) collected 74 indoor gas phase air and 30 household dust samples from kindergarten and apartments in Berlin. Results of the indoor air sampling in kindergarten showed that HHCB had the highest concentration on average of 101 ng/m^3 (range:15–299 ng/m^3) followed by AHTN and AHMI. Researchers did not detect DPMI and MX in any of the samples. MK, ATII, and ADBI were found in a few samples with maximum level of 12 ng/m^3, 17 ng/m^3 and 34 ng/m^3 respectively.

The household dust samples only HHCB, AHTN and MK were observed. ADBI, ATII, AHMI, DPMI and MX were under the detection limit. HHCB was observed in 19 samples while AHTN was observed in 25 samples. MK was only in 11 samples from 30 household dust samples. The maximum values were detected 11.4 ng/m^3, 3.1 ng/m^3, and 47 ng/m^3 for HHCB, AHTN and MK respectively.

Chen et al. (2007) studied the concentration and distribution of polycyclic musks DPMI, ADBI, AHMI, AHTN, and HHCB in a cosmetic plant air in the HuangPu industrial park in GuangZhou. Air was sampled four different site (ID-in the work shop of cosmetic plant, M-out of the workshop of the cosmetic plant, OD-download direction about 200m away from the cosmetic plant, TH-upwind direction about 25 km away from the cosmetic plant) with both particulate (GFF, 20.3cm x 25.4 cm) and gas phase (PUF). The highest concentrations were detected for HHCB and AHTN in ID gas phase samples, approximately 4505 ng/m^3 and 725 ng/m^3. Synthetic musks commonly detected in gas phase at the percentage of 86.35 to 97.70 % whereas particulate phase concentrations were detected very low. The highest concentration was 100 ng/m^3 in particulate phase for HHCB. The descending orders of concentrations were detected through ID, M, OD, and TH. The diffusion or dilution of musks in the air was attributed to this order. The authors found out that the synthetic compounds were also detected both ID and TH locations, therefore they concluded that cosmetic plants would be an important environmental pollution source of polycyclic musks.

2.3. Risk assessment

Due to potential negative effects of these compounds to the environment, human beings, animals, biota and even microorganisms, risk assessment studies are crucial to assess the relative importance of each contaminant. Ecological risk assessment has been carried out by comparing the measured or estimated environmental exposure concentrations (PEC) with known or predicted no effect concentration (PNEC). If the ratio of PEC/PNEC (Risk Quotient, RQ) is <1, then the risk of harm to the environment is assumed insignificant (SWEDISH SOC. NAT. CONS. FOUN. 2009). The ecological risk levels were reported for aquatic organisms and organisms in sediments and soil where musks commonly detected at high levels.

Swedish Society for Nature Conservation reported a wide-range of risk assessment for MX and MK (Tas et al., 1997). The RQ was reported as <1 for aquatic organism and organisms in sediments for both MK and MX. In this report, PNEC and PEC had been calculated assuming ten yearly applications of sludge to agricultural land. The RQ had been found just above one for the soil. On the other hand, according to recent sludge concentration data, the levels below the demarcation value and sometimes detected below the detection limit. Thus, the risk quotient for soil assumed was to be <1 for both MK and MX by Tas et al. (1997).

Moreover, Ecotoxicological Assessment Criteria (EACs) may be used to evaluate environmental musk ketone and musk xylene concentrations to report concentrations below EACs as safe. EAC values ranged 0.01 to 0.1µg/L for water, 5 to 50 mg/kg for sediment, 0.5 to 5 mg/kg for mussel, and 0.5 to 5 mg/kg for fish for musk xylene. EAC values were reported for musk ketone 0.05 to 0.5µg/L for water, 1 to 10 mg/kg for sediment, 0.1 to 1 mg/kg for mussel and 0.1 to 1 mg/kg for fish (Swedish Soc. Nat. Cons. Foun., 2009).

A similar risk assessment has been reported for polycyclic synthetic musks. The RQ was <1 for aquatic organism and sediments for HHCB and AHTN. Similar to MX, the soil RQ was estimated as >1. However, sludge levels smaller so the RQ was estimated as <1. Moreover, because of smaller volume of usage , low risk levels were expected for aquatic organisms and sediment organisms for AHMI, ATII and ADBI.

The Scientific Committee on Cosmetic and Non-Food Products (SCCNFP) reported toxicological evaluations and risk assessments MX and MK. They have taken Lehmann-McKeeman et al. (1997) as a guide for the determination of the human health risks. SCCNFP reported that MK and MX are weak estrogens and persistent in the environment, therefore, they can be classified as category 3 carcinogens (SCCNFP, 2004).

Lehmann-McKeeman et al. (1997) studied induction and inhibition of mouse cytochrom P450 2 B enzymes by musk xylene. Researchers dosed musk xylene through gavages to mice for 7 days at 0, 1, 5, 10, 20, 50, 100 and 200 mg/kg. Musk xylene caused significant inhibitory effect on growth in high dose groups with 170 mg/kg of body weight/day; this situation was observed from 4 weeks to 80 weeks. The increased tumor incidences were reported for liver and harderian gland. MX significantly increased liver weights up to dosages of 20 mg/kg and higher while reaching a maximum of 65% increase at 200 mg/kg. However, up to the 10 mg/kg they did not observe enzyme induction and inhibitation in mice. Therefore, SCCNFP assumed 10 mg/kg.bw/d might be considered as a no observed adverse effect level (NOAEL). Based on this data, SCCNFP calculated and reported lifetime cancer risk for mice and extrapolation to humans. 7.3 µg/kg bw/d dose represents a lifetime cancer risk of 10^{-4} when liver carcinomas or harderian gland tumors was taken into account as a base and lifetime exposure dose. 22 µg/kg bw/d was reported as the worst case daily intake of MX and the life time cancer risk was reported as about $3x10^{-4}$. SCCNFP concluded that both MX and MK have low acute subchronic toxicity. However, MX was reported as carcinogenic, liver cell tumors were clearly increased in mice when MX was dosed. 10 µg/kg bw/d was suggested NOAEL for musk xylenes.

Furthermore Swedish society for nature conservation reported the lowest chronic toxicity (NOEC) values and acute toxicity values (half maximal effect concentrations (EC_{50}) and lethal concentration (LC_{50})) for use in risk assessments (Swedish Soc. Nat. Cons. Foun., 2009). No effect concentration (NOEC) refers to largest concentration that the test organism reproduction or growth is not significantly different with respect to control organisms (Pires et al., 2002). EC_{50} is commonly used as a measure of drug potency and toxicity, and refers to concentration of a drug or toxicants in an environment that is expected to affect 50% of test organisms in a specified exposure time, while LC_{50} refers to a concentration that is expected to kill 50% of organisms under a defined conditions (IUPAC, 1993). The reported NOEC values are: 0.063 mg/L for MK, 0.068 mg/L for HHCB and 0.035 mg/L for AHTN for fish and 0.056 mg/L for MX for Daphnia. The aquatic toxicity for the nitro musks and polycyclic musks are summarized in Table 2.3 and Table 2.4, respectively (Swedish Soc. Nat. Cons. Foun., 2009). No toxicity data could be obtained from the literature for the inhalation route of the exposure.

The SCCNFP study was taken as guidance for the risk assessment in this study. Average daily dose (ADD) is estimated with a general formula in equation 1 (EPA, 1997) and the risk estimates were calculated based on reported the lifetime cancer risk of 10^{-4} at 7.3 µg/kg /day. As follows; lifetime cancer risk levels are calculated in the result chapter.

$$ADD = [[C \times IR \times ED] / [BW \times AT]] \quad (2.1)$$

Where ADD = Average Daily Dose (µg/kg/day); C = Contaminant Concentration in Inhaled Air (µg/m^3); IR = Inhalation Rate (m^3 /hr); ED = Exposure Duration (hr); BW = Body Weight (kg); and AT = Averaging Time (days).

Table 2.3. Aquatic toxicity for nitro musks
(Source: Swedish Soc. Nat. Cons. Foun. 2009)

Nitro musks	Bacteria	Algae	Crustacean	Fish
Moskene	Vibrio fischeri EC_{50}^{*} 30 min. > 0.037 mg/L	Scenedesmus, subspicatus EC_{50} 72h > 0.046 mg/L (no effect at water solubility)	Daphnia magna, EC_{50} 48h > 0.046 mg/L (no effect at water solubility)	
Musk Tibetene	Vibrio fischeri EC_{50} 30 min. > 0.042 mg/L	Scenedesmus subspicatus, EC_{50} 72h > 0.052 mg/L (no effect at water solubility)	Daphnia magna, EC_{50} 48h > 0.052 mg/L (no effect at water solubility)	
Musk Ketone	Vibrio fischeri EC_{50} 30 min. > 0.34 mg/L	Selenastrum capricornutum, EC_{50} 72 h = 0.244 mg/L Scenedesmus subspicatus, EC_{50} 72h > 0.46 mg/L (no effect at water solubility)	Daphnia magna, EC_{50} 48h > 0.46mg/L (no effect at water solubility) Daphnia magna, EC_{50} 21d (reproduction) =0.169-0.338 mg/L	Oncorhynchus mykiss, NOEC 21 d. = 0.063 mg/L
Musk Xylene	Vibrio fischeri EC_{50} 0.30 min. > 0.12 mg/L	Selenastrum capricornutum, NOEC 5d. > 5.6 mg/L Scenedesmus Subspicatus, EC_{50} 72h > 0.15 mg/L (no effect at water solubility)	Daphnia manga, NOEC 48h. = 0.32 mg/L Daphnia manga, NOEC 21d (re-production) = 0.056 mg/L	Bluegill sun fish, LC_{50}^{**} 96h =1.2 mg/L Brachydanio rerio, LC_{50} 14d. = 0.4 mg/L

Table 2.4. Aquatic toxicity for polycyclic musks
(Source: SWEDISH SOC. NAT. CONS. FOUN. 2009)

Polycyclic musk	Algae	Crustacean	Fish
AITI		Daphnia magna, EC_{50} 48h. = 0.42 mg/L	
AHMI	Selenastrum capricornutum, EC_{50} 72h. = 0.081 mg/L EC_{50} 72h. = 0.2 mg/L NOEC (growth rate) = 0.044 mg/L	Daphnia magna, 48h. = 0.33 mg/L EC_{50}	Brachydanio rerio NOEC 96h. = 0.9 mg/L
HHCB	Pseudokirchneriella subcapitata, EC_{50} 72 h = 0.723 mg/L	Daphnia magna, NOEC 21d. = 0.111 mg/L	Lepomis macrochirus, NOEC 21d. = 0.182 mg/L Pimephales promelas, NOEC 36d. = 0.068 mg/L
AHTN	Pseudokirchneriella subcapitata EC_{50} 72 h = 0.468 mg/L	Daphnia magna NOEC 21d. = 0.196 mg/L	Lepomis macrochirus, LC_{50} 21d. = 0.314 mg/L Pimephales promelas, NOEC 36d. = 0.035 mg/L

CHAPTER 3

EXPERIMENTAL STUDY

In this study gas and particulate phase samples collected in Izmir to obtain the synthetic musk fragrances HHCB, AHTN, ATII, ADBI, AHMI, DPMI, MX, and MK. 10 gas and particulate phase samples were collected from primary school during November 2008, and 10 samples gas and particulate phase were collected sport centers between June and July 2009. The Primary School is located in urban area and sport center is located suburban area where they were under the effect of synthetic musks via cleaning agents and personal care products. The frequency of the cleaning was on regular daily basis for both primary school and sport center. Sport center has 135 m^2 area with a average population density 5 person per hour. The building has 2 doors on the front and back of the building. Primary school has 3 buildings which constructed in 1968, 1992 and 1988. The total area of buildings are 930, 130 and 350 m^2 and school has 1858 occupants that constitute of 1760 students, 86 teachers, 8 janitors and 4 canteen workers. The occupant density was 0,68 student/m^2/hr for the sampling classroom in primary school.

3.1. Preparation

For all cleaning and extraction procedures MilliQ water (Millipore Elix 5) and high purity solvents (Merck (SupraSolv), GC grade) were used. The cleanup procedure had been conducted in three parts separately: glassware, metals and filters.

3.1.1. Glassware

All glassware was washed with Alconox with hot water followed by distilled water. Then, they were rinsed with acetone and hexane, which are polar and nonpolar solvents respectively. They were dried in an oven at 110 ^{0}C for 4 hour. Then, glassware was wrapped with aluminum foil and kept in oven till use.

3.1.2. Metals

All metal apparatus like tweezers and spatula were washed with Alconox and hot water followed by distilled water. Then, they were dried in an oven with 60 ^{0}C for 3 hours. Then, the metals were wrapped with aluminum foil.

3.1.3. Filters

3.1.3.1. Polyurethane Foam Filters (PUF)

PUF filters were cleaned by Soxhlet extraction using dichloromethane and acetone-hexane mixture. A picture of the Soxhlet extraction apparatus is shown in Figure 3.1. First of all filters were extracted by using dichloromethane for 6 hours. Then, extraction followed by using acetone-hexane (1:1) mixture for 8 hours. Then, PUF filters were wrapped aluminum foil and dried at 60 ^{0}C for 3 hours in an oven.

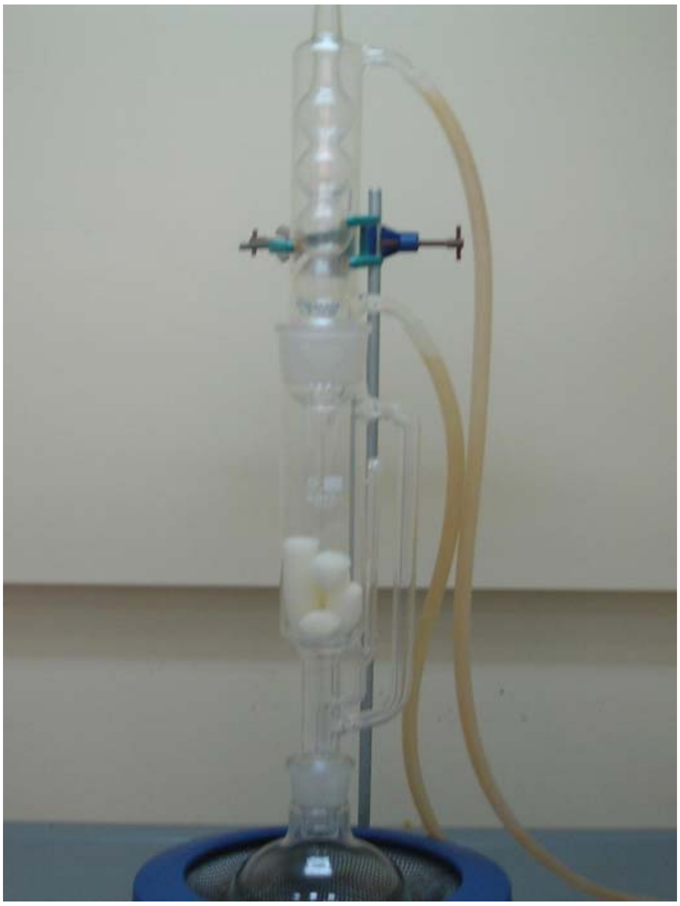


Figure 3.1. Soxhlet extraction apparatus

3.1.3.2. Glass Fiber Filters

Glass fiber filters were wrapped with aluminum foil and baked overnight at 450 ^{0}C. Then, they were stored and cooled in a dessicator until use.

3.1.3.3. Metal Filters

Like other metal cleanup procedure, metal filters firstly washed with Alconox with hot water for 30 minutes. Than they were rinsed water again and placed in beaker.

The beaker filled with water and overflowed. The metal filters were dried at 60 ^{0}C for 3 hours and wrapped with aluminum foil.

3.2. Air Sampling

Air samples were collected with using active air sampling system as shown in Figure 3.2. This system consists of mainly three parts: impactor, polyurethane foam cartridge and pump. The impactor contains the sampling inlet apparatus, the particle impactor which is the metal filter and has 2.5 µm size cut, and filter holder where 37 mm glass fiber filters (1 µm pore) places into for collecting the airborne particles ($PM_{2.5}$). The impactor is connected to polyurethane foam (PUF) cartridge. PUF (22 mm diameter and 100 mm length) is placed in hollow cylinder with glass cylinder. The PUF cartridge is connected to a vacuum pump. Gas phase sampling media is PUF while glass fiber filter (GFF) or quart can be used for particulate phase.

Air firstly passes through the impactor which consists of glass fiber filter. In this way airborne particles were collected with filters for detecting $PM_{2.5}$ and particulate phase concentrations. Then air passes through the PUFs cartridge which consists of PUFs. Finally air passes through the air vacuum pump and ventilates in ambient air.

Gas and particulate phase samples were collected for 8 hours with a 10 L/min flow rate in primary school, and for 12 hours with 6.5 L/min in sport center. Average total volumes of all samples were nearly 4.8 m^3 air. In each sampling before sampling, the pump was calibrated with flow meter. At the end of sampling the flow rate was measured for calculation of daily average flow rate. Moreover, all glass fiber filters were weighted before and after sampling for determination of the particulate matter ($PM_{2.5}$) concentration.

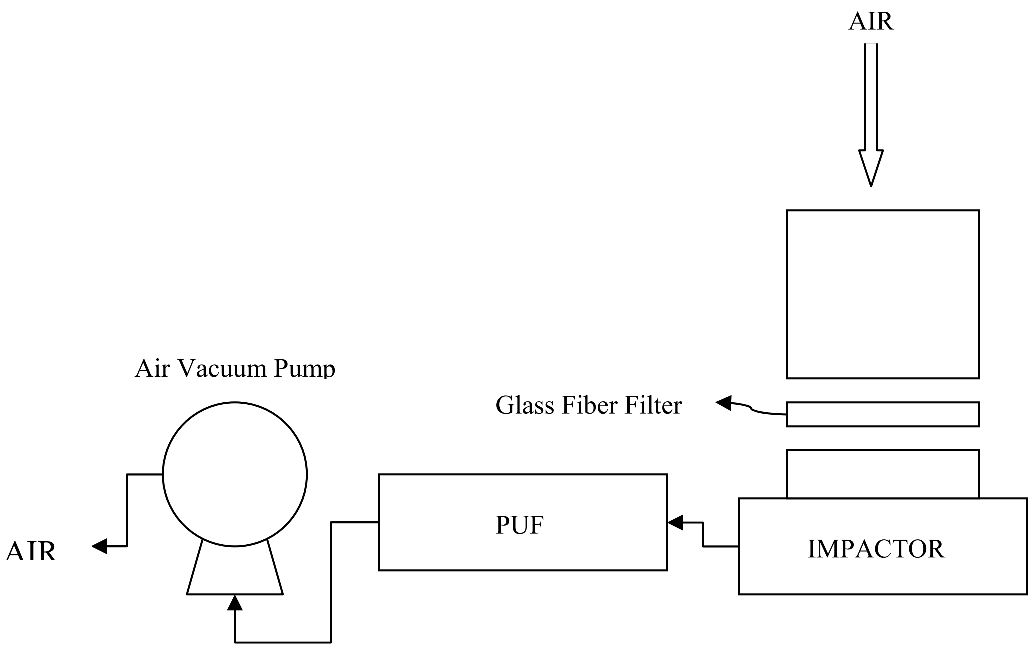


Figure 3.2. Air Sampling System

3.3. Extraction, Concentration and Cleanup

3.3.1. Extraction of Poly Urethane Foams (PUF)

Before the extraction procedure with a known concentration (50 pg/µl), 10 µl fluoranthene-d_{10} (AccuStandard) was added each vials for recovery as an internal standard. PUF and filters were placed in 40 ml amber vials and 20 ml acetone and 20 ml hexane solvents (1:1) were added. Then, the vials inserted into ultrasonic extraction (Elma) tray for one hour. Then PUF and filters were taken from vials to continue the rest of the procedure.

3.3.2. Concentration

The sample extracts were evaporated ~ 5 ml volume with gently blowing nitrogen as shown in Figure 3.3. Then for the solvent exchange, 10 ml hexane added and evaporated ~ 5 ml volume, and this procedure performed for twice and finally sample extractions volume was brought up to 2 ml.

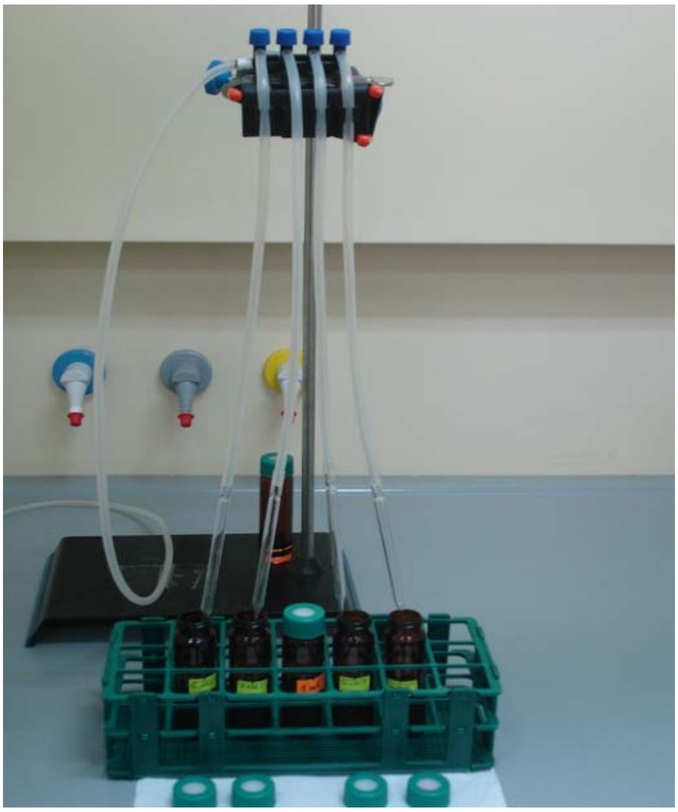


Figure 3.3. Evaporation apparatus

3.3.3. Sample Cleanup

After concentrating all sample extracts were passed through a chromatography column to enrich the compound of interest and eliminate of the interfering organic compounds. A picture of the column is shown in Figure 3.4. The column was filled

0.75 g 100-200 mesh Florisil (Sigma-Aldrich) and topped with 1 cm anhydrous sodium sulfate to get rid of any water residue. Florisil, is a magnesium silicate with basic properties, has been used for clean up of pesticide residues, PCBs, PAHs, chlorinated hydrocarbons, aromatic compounds, fat, oil, waxes and separation of nitrogen compounds from hydrocarbons (EPA, 2007). Florisil activated with baked at 650 ^{0}C and sodium sulfate activated at 450 ^{0}C for overnight. Then, cooled and stored in desiccators at room temperature. For the deactivation of Florisil 37.5 µl water added for 0.75 g Florisil before filled up to the column.

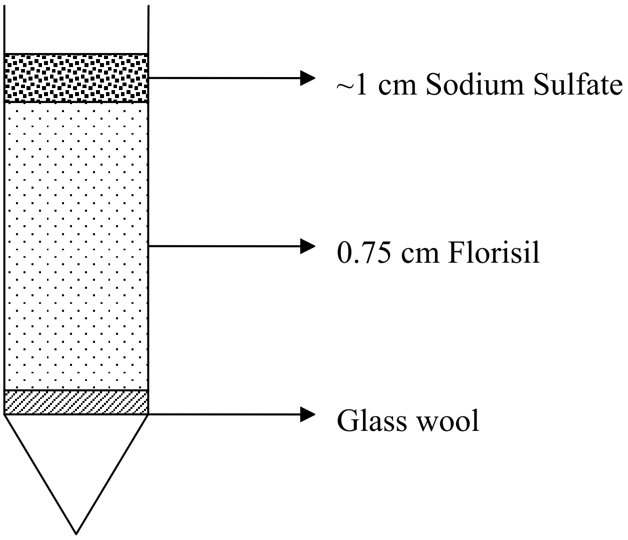


Figure 3.4. Florisil Column

Sample passed through the column and the eluate discharged. Then 4 ml ethyl acetate (Merck (SupraSolv), GC grade) passed through the column and the eluate was collected in 40 ml amber vial.

The collected eluent were evaporated again and their solvent exchanged to the hexane. In this concentration step, final sample volume was reduced ~ 1 ml with using nitrogen. Then samples were taken 2 ml amber vials that shown in Figure 3.5 and stored at -20 ^{0}C up to GC-MS analysis.

Figure 3.5. Samples Stored in Amber Vials

3.4. GC-MS Analysis

Thermo Trace GC Ultra gas chromatograph coupled to Thermo DSQII mass selective detector with electron impact ionization (GC/EI-MS) operating in selected ion monitoring (SIM) mode was used for the analysis of synthetic musks. A 30-m 5% phenyl methyl siloxane capillary column was used (HP-5MS; 250 µm I.D., 0.25 µm film thickness). Samples were injected programmable temperature vaporizer (PTV) splitless modes. A constant 1 ml/min column flow rate was used. The oven temperature, which is shown in Figure 3.6, was held for 1 min in 60 ^{0}C. Then temperature was ramped from 60 to 180 ^{0}C at 15 ^{0}C/min followed by a 0.2 ^{0}C/min ramp to 185 ^{0}C. The final temperature (290 ^{0}C) was held for 6 min. The slow temperature gradients are necessary for the simultaneous analysis of the synthetic musk fragrances and polycyclic aromatic hydrocarbons. The MS transfer line temperature was 300 ^{0}C. Each synthetic musk fragrance was quantified using its most abundant ion (quantification ion). The compounds were identified by retention time. Table 3.1 shows that obtained synthetic musk fragrances retention time and ions.

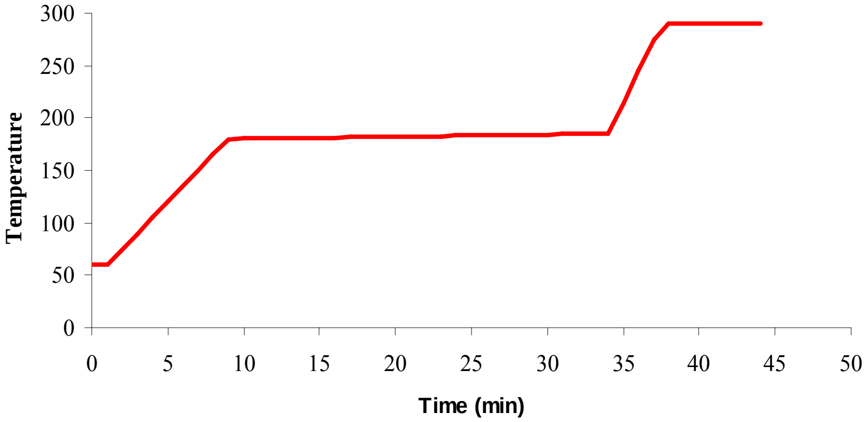


Figure 3.6. GC-MS Oven Temperature Program

Table 3.1. Synthetic Musk Fragrances Retention Time and Ions

Compounds	Retention time (min)	Target ion (m/z)	Reference ion (m/z)
DPMI	9.51	191	206
ADBI	12.15	229	244
AHMI	12.92	229	244
ATII	14.88	215	258
HHCB	14.97	243	213
MX	15.21	282	297
AHTN	15.27	243	258
MK	19.07	279	294
fluoranthene-d_{10}	21.77	212	

3.5. Quality Assurance/ Quality Control

Sample collection efficiency can be analyzed by determining the important parameters for sample collection with the samplers used in the study. In general, areas of concern in the sampling are adsorption of gas phase on filter, breakthrough and volatilization for SVOC's sampling with PUF. The researchers usually reported that around 200-300 m^3 sample volumes, the breakthrough on the back up plug in this volume was 11 % of the front plug (real sample). At reasonable volumes, they mentioned that breakthrough could not be observed. In our study, the volume is 5 m^3 which is too low to observe significant breakthrough. To check the breakthrough 4 back-up samples from primary school classroom and 3 back-up samples from Sports Center were collected into a second PUF serially connected, called as back-up plug, to the system during the sampling. All synthetic musk compounds were detected on the back-up plugs except musk ketone and AHMI. Detected average back-up sample concentrations and distribution with back-up and real samples are listed in Table 3.2. In both sampling station DPMI was detected as the highest among the others. 50 % of the concentration of DPMI was observed in the back up plugs. The rest of the compounds of the amounts seen in the 20 % and less. It can be clearly seen that primary school classroom samplings were more efficient than sport center. Higher retention was determined in the first PUF (real sample). Due to difference in the sampling time could cause volatilization from sampling media. Therefore the sampling of synthetic musk should be combined with resin and PUF.

The polyurethane foams and glass fiber fibers were treated as real samples to determine any contamination of synthetic musk compounds as well field blanks. The same preparation and clean up procedures were followed as applied to real samples.

Three laboratory blanks and field blank were analyzed for glass fiber filters. Particulate matter blank ($PM_{2.5}$) weight was found 200 µg. The minimum detected concentration values for each synthetic musk is reported in Table 3.3 .

Table 3.2. % Retention of the Front Plug (Real Sample)

Compounds	% Retention of Sports Center	% Retention of Classroom
DPMI	49.33	48.56
ADBI	79.80	86.24
AHMI	-	-
ATII	76.87	92.62
HHCB	76.64	92.22
MX	88.65	96.67
AHTN	84.04	95.18
MK	-	-

n.d. = not detected

Table 3.3. Glass Fiber Filter Blanks (ng) and Detection Limits (ppb-pg/µl)

Compound	Laboratory blank	Field blank	Detection limit
DPMI	5.25±0.9	n.d.	1
ADBI	n.d.	n.d.	0.05
AHMI	n.d.	n.d.	0.02
ATII	0.32±0.1	2.5	0.2
HHCB	2.70±0.2	11.9	0.1
MX	n.d.	n.d.	0.2
AHTN	0.97±0.2	15.5	0.1
MK	n.d.	n.d.	1

n.d. = not detected

This might be interpreting the glass fiber filters were contaminated during carriage and laboratory conditions. However, except DPMI all detected compounds were higher in field blank. This shows that the glass fiber filters were contaminated higher during carriage and contaminations was less due to preparation and clean up procedure.

3 laboratory blanks and field blank was taken for PUFs and analyzed like real samples PUFs procedure. Average masses of blanks for PUFs are listed in Table 3.4. Like glass fiber filters blanks synthetic musks are detected in PUFs laboratory and field blanks. For the gas phase, both laboratory and field blank concentration values are detected similar values. Only ATII, HHCB and AHTN compounds are detected in laboratory blanks with 4.97 ng, 1.97 ng and 7.95 ng respectively. Likewise only ATII, HHCB and AHTN compounds were detected in field blanks with 4.12 ng, 2.35 ng and 5.85 ng respectively.

Table 3.4. Polyurethane Foam Blanks (ng)

Compound	Laboratory blank	Filed blank
DPMI	n.d.	n.d.
ADBI	n.d.	n.d.
AHMI	n.d.	n.d.
ATII	4.97±0.3	4.12
HHCB	1.97±1.7	2.35
MX	n.d.	n.d.
AHTN	7.95±0.5	5.85
MK	n.d.	n.d.

n.d. = not detected

These blank results show that like glass fiber filters also PUFs were contaminated during carriage and clean up procedure. Because of synthetic musks were detected from blanks without sampling, it should be refer that special care has to be consumed for sample preparation, carriage, clean up and other laboratory steps.

3.5.1. Method Recovery

For the method recovery evaluation flouranthene-d_{10} were used as surrogate standard. It was added at the beginning of the extraction process. The concentrations were corrected according to flouranthene-d_{10}. In addition to this spike recovery procedure was conducted for gas and particulate phase. The standard mixture with a defined concentration containing all synthetic musk compounds was spiked on to PUF and filter. Then these spike recovery samples were treated as real sample. They were analyzed with GC-MS. The percentages recoveries for each substance are listed in Table 3.5. The particulate phase recoveries were between 68.5 % to 83.4 %, and the gas phase recoveries were changed between 44 % to 54.2 %. The values for the gas phase were quite low. These recovery results are similar to Chen et al. (2007) results in literature, where the mean recoveries (%) were detected between 48.12 to 81.36 %.

Table 3.5. Spike Recovery Results

Compounds	Gas Phase Recovery (%)	Particulate Phase Recovery (%)
DPMI	44	68.5
ADBI	49.8	73.4
AHMI	46.8	74.5
ATII	49.9	80.3
HHCB	47.5	81.5
MX	48.2	72
AHTN	50.1	77.8
MK	54.2	83.4

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Particulate Matter ($PM_{2.5}$) Concentrations

Ten gas and particulate samples were collected in the primary school classroom in the autumn while ten gas and particulate samples were collected from sports center in summer. For the gas-phase PUFs were used and particulate matters ($PM_{2.5}$) were collected on the glass fiber filters.

Table 4.1 and 4.2. show the average daily total volume used for sampling $PM_{2.5}$ concentrations were also measured and shown in the tables. World Health Organization (WHO) reported that adverse health effects occurrence limit for $PM_{2.5}$ is 10 $\mu g/m^3$ for annual mean and 25 $\mu g/m^3$ for 24 hour mean (WHO, 2005).

Table 4.1. $PM_{2.5}$ Concentrations and Average Air Volume in the Primary School Classroom

Sample ID	Air volume (m^3)	$PM_{2.5}$ (g)	$PM_{2.5}$ ($\mu g/m^3$)
G0411	5.04	0.0012	232.07
G0511	5.1	0.002	389.06
G0611	5.04	0.0012	237.02
G1011	4.8	0.0018	372.60
G1111	4.98	0.0013	261.36
G1211	5.04	0.0031	607.07
G1311	4.86	0.0021	432.99
G1811	4.98		
G1911	5.04		
G2111	5.04		

Table 4.2. $PM_{2.5}$ Concentrations and average daily total air volume in Woman Sports Center

Sample ID PUF	Air volume (m^3)	$PM_{2.5}$ (g)	$PM_{2.5}$ ($\mu g/m^3$)
U1606	5.05	0.0004	79.14
U1806	4.59	0.0002	43.60
U2306	4.79	0.0004	83.52
U2406	4.77	0.0003	62.86
U2506	4.77	0.0005	104.75
U2906	4.84	0.0002	41.33
U3006	4.19	0.0003	71.68
U0107	4.44	0.0005	112.49
U0207	4.76	0.0002	42.00
U0607	4.47	0.0004	89.44

The particulate matter concentrations were higher for primary school than sports center. This could be due to high activity and high population in the classroom. Diapouli et al. (2007) were measured the highest indoor particulate matter ($PM_{2.5}$) about 200 $\mu g/m^3$ in the highly populated area school in a year study. The average values for the seven schools were 82.6 $\mu g/m^3$ which is close to sports center value measured here.

4.2. Particulate Phase Concentrations

Figure 4.1, Figure 4.2, and 4.3 illustrate the particulate matter concentrations for Primary School and Sports Center and the comparison of the concentrations respectively. The detected musk concentrations of primary school were higher than Sports Center similar to the measured particulate matter concentrations. In primary school five of eight musk compounds (ADBI, ATII, HHCB, MX and AHTN) were detected. The highest concentrations were determined for HHCB and AHTN while the lowest compounds ADBI, followed with ATII, MX.

In the Sports Center only three musk compounds (HHCB, ATII and AHTN) were detected. The only study conducted in the particulate phase concentration was in the cosmetic plant particulate phase concentration in China. The measured particulate phase concentration for the total musk was 127.4±41 μg/m^3 in the workshop of the plant. However, the other sampling point concentrations were lower where the sampling locations were outside of the workshop, 200 m away from the plant downwind direction and 25 km away from the plant upwind direction and the concentrations were 6.39 ±1.35, 2.35 ±0.25 and 1.71 ±0.16 μg/m^3 respectively. Due to the decrease in the gas phase concentration, they found out the contribution of particulate phase increased at all these sampling points (Chen et al, 2007).

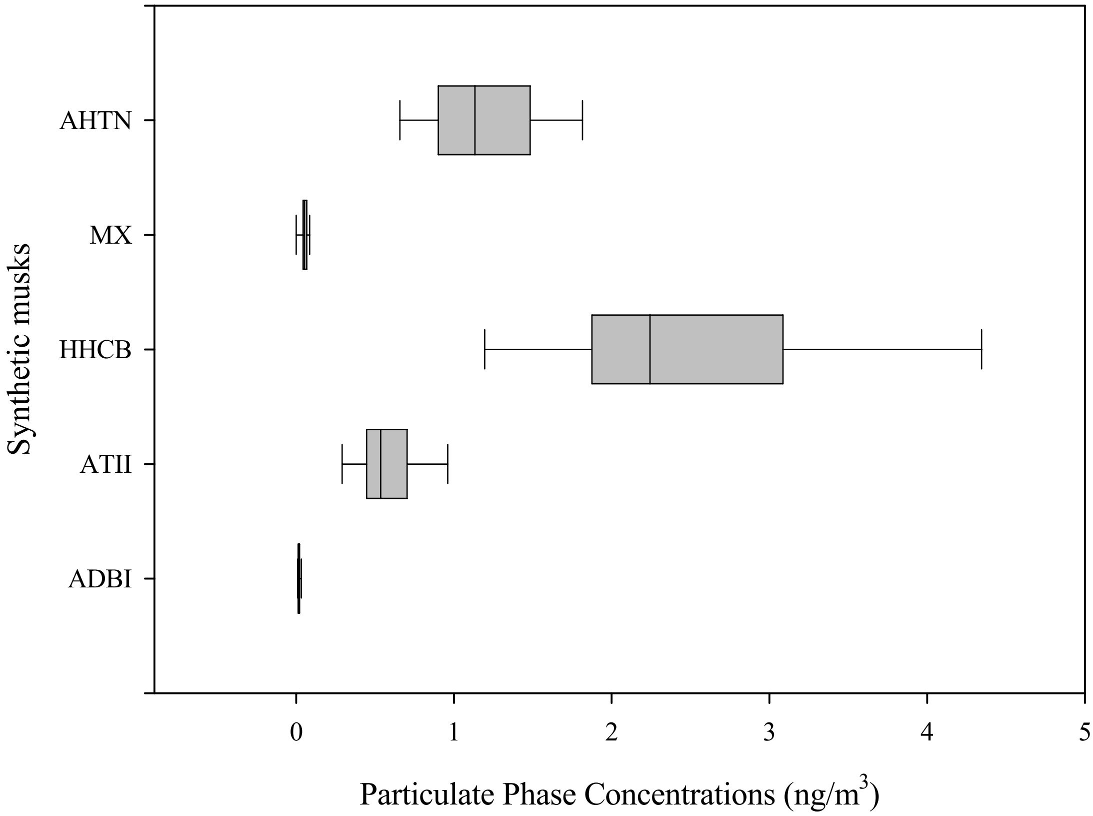


Figure 4.1. Primary School Particulate Phase Concentrations (ng/m^3)

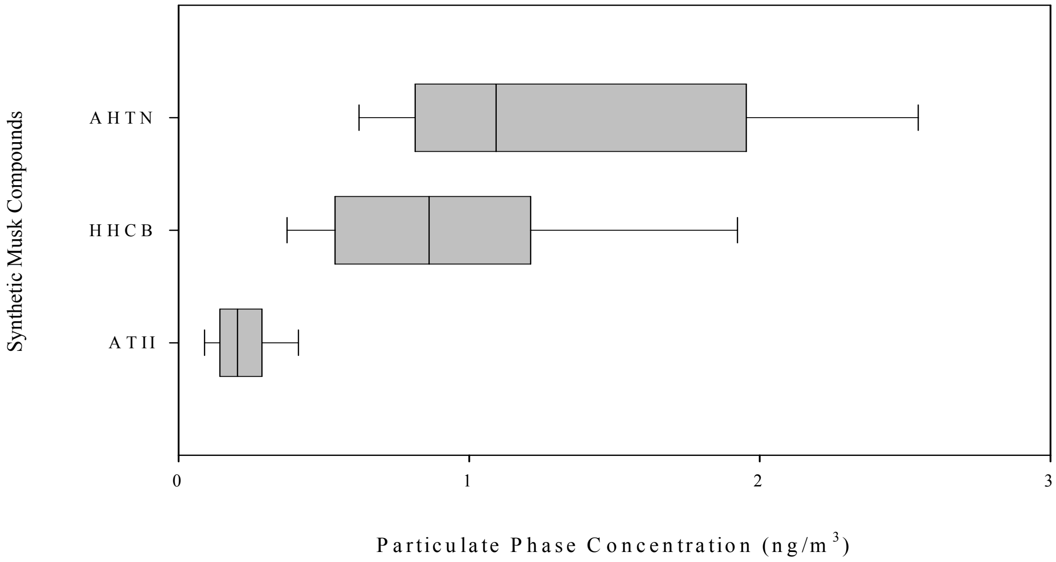


Figure 4.2. Sport Center Average Particulate Phase Concentrations (ng/m^3)

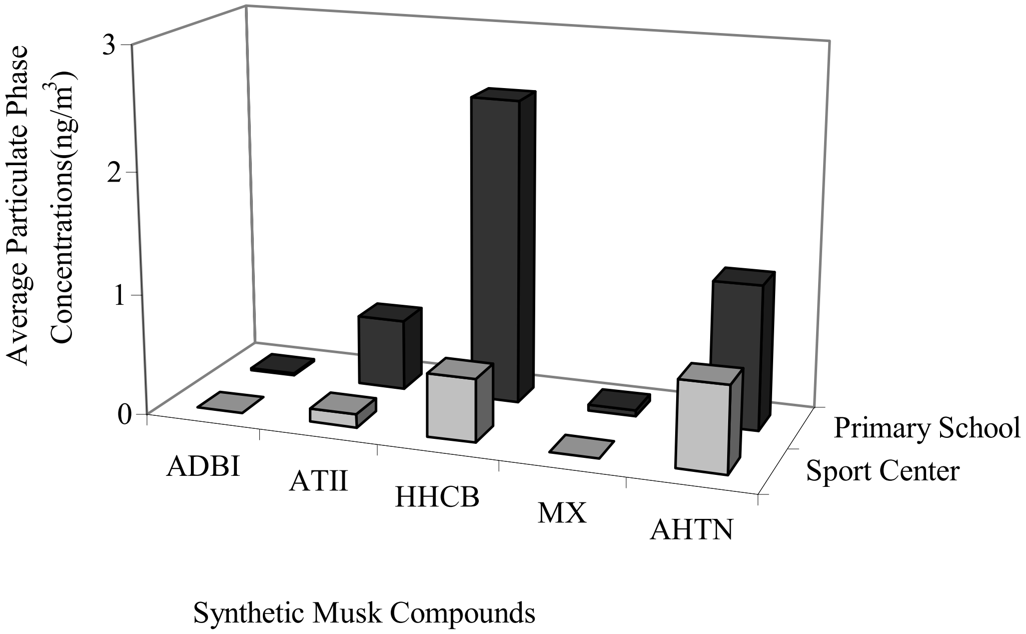


Figure 4.3. Comparison of Average Particulate Phase Concentrations between Sports Center and Primary School

4.3. Gas Phase Concentrations

The gas phase concentrations for primary school ranged from 267±56 (HHCB) to 0.12±0.2 ng/m^3 (MK) while it varied from 144±60.6 (HHCB) to 0.08±0.1 ng/m^3 (AHMI) for Sports Center. From the highest to lowest concentration order is HHCB>AHTN>ATII>DPMI>MX>ADBI>AHMI for Sports Center while it is HHCB>DPMI>AHTN>ATII>MX>ADBI>AHMI>MK for primary school classroom respectively. Musk Ketone was not detected in Sports Center at all. The dominant compound was HHCB in the both sampling places.

Fromme et. al. (2004) studied in 59 apartments and 74 kindergartens in Berlin in the year of 2000 for the presence of phthalates and musk fragrances, polycyclic musks in particularly. For the 74 air measurements in the kindergartens HHCB gave the highest levels with an average, 101 ng/m^3 (range: 15–299 ng/m^3). HHCB with 61% made the greatest contribution to the total measured musk componds followed by AHTN (26%) and AHMI (12%). This relates closely to present day application of these substances. DPMI and musk xylene could not be measured in any of the samples. MK and ATII were found in a few samples at levels from 12 ng/m^3 to a maximum of 17 ng/m^3.

Chen et al. (2007) measured the concentrations of polycyclic musks (Cashmeran (DPMI), Celestolide (ADBI), Phantolide (AHMI), Traseolide (ATII), Tonalide (AHTN) and Galaxolide (HHCB)) in the air, and other medias in a typical cosmetic plants and this study was the only study reporting indoor air concentrations of gas phase of synthetic musk compounds. The highest concentrations were detected as major compounds HHCB and AHTN in the sites. The total concentration of polycyclic musks in the study was 5416.07 ± 1079.11 ng/m^3 and 96.5 % of contribution was coming from HHCB and AHTN. It is not forgotten this value is belong to the workplace. However, the authors measured the concentrations around the plant and the levels were also high and they concluded that the levels could affect habitants around the plants.

In our study, in both sampling area the most detected compounds are similar with literature except for DPMI. This is due to the common use of these compounds in household and personal care products. The least detected compound was determined in our samples implying the use in the products.

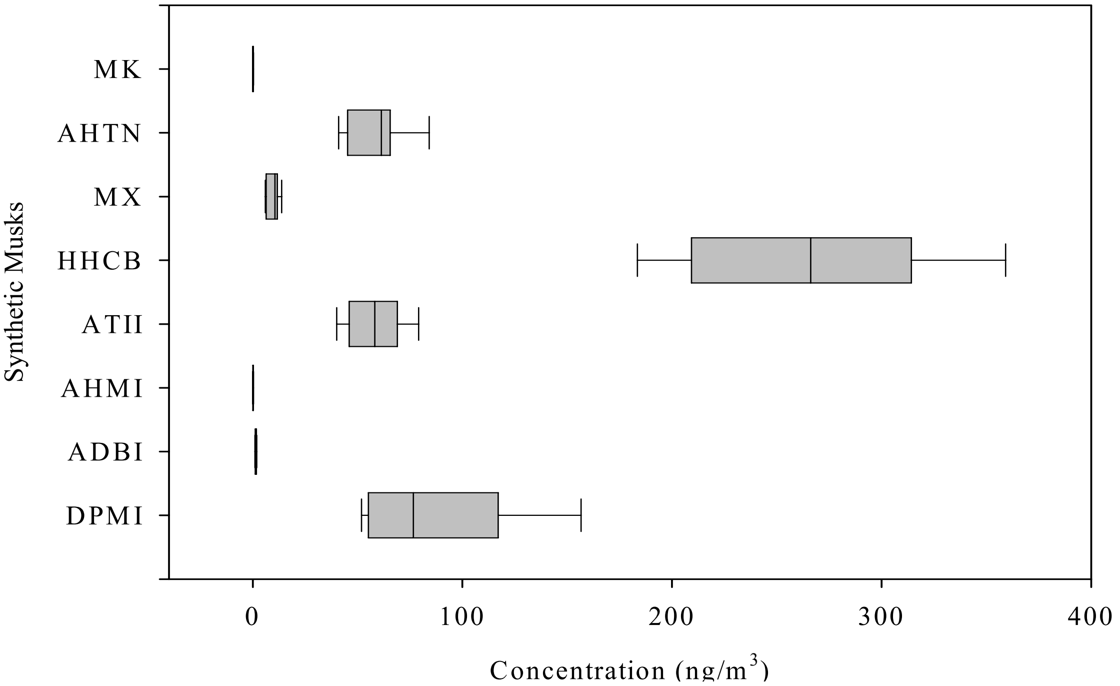


Figure 4.4. Primary School Gas Phase Concentrations (ng/m^3)

The classroom gas phase concentrations were higher than sports center as seen in Figure 4.4 and Figure 4.5. However the gas phase concentrations detected in the classroom were ranged 0.11-298 ng/m^3 which was similar to the Berlin study (Fromme et al., 2003). Fromme and coworkers detected the gas phase concentrations between 22-119 ng/m^3 in the kindergartens. The difference between two sampling places may be caused higher population density in the classroom than sports center. Moreover the other reasons would be cleaning products strength, cleaning duration, natural ventilation frequency, and season difference in the sampling time.

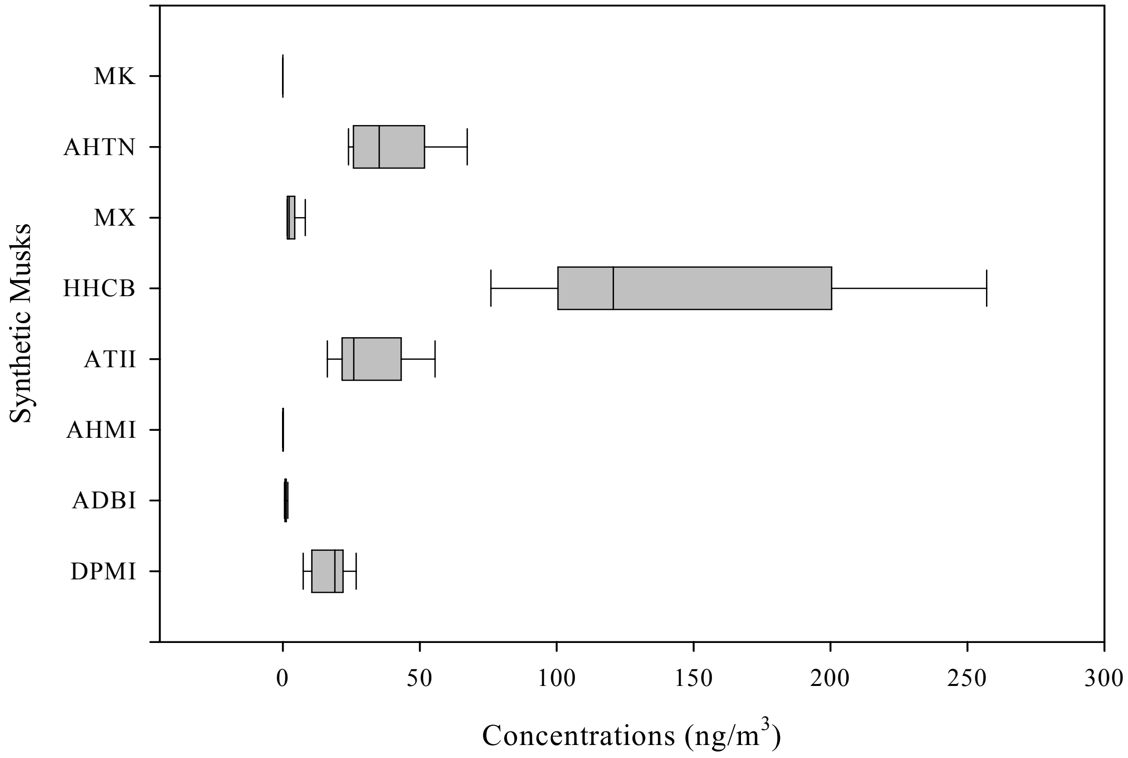


Figure 4.5. Sport Center Gas Phase Concentrations (ng/m^3)

4.4. Gas/Particle Phase Distributions

Gas/particle phase (G/P) distribution showed that the synthetic musk compounds tendency of being either gas or particulate phase in the indoor air. Gas/total phase concentration ratio was used to plot the Figure 4.6. and 4.7. Distribution of G/P phase showed that all of the synthetic musks were present above the 96 % in the gas phase in sport center while it was 98 % and above in the classroom.

In the Figure 4.6 can be clearly seen that the gas phase distributions are higher than particulate phase. Only three compounds, HHCB, AHTN and ATII, have been determined on the particulate phase the others were 100 % on the gas phase at the Sport Center.

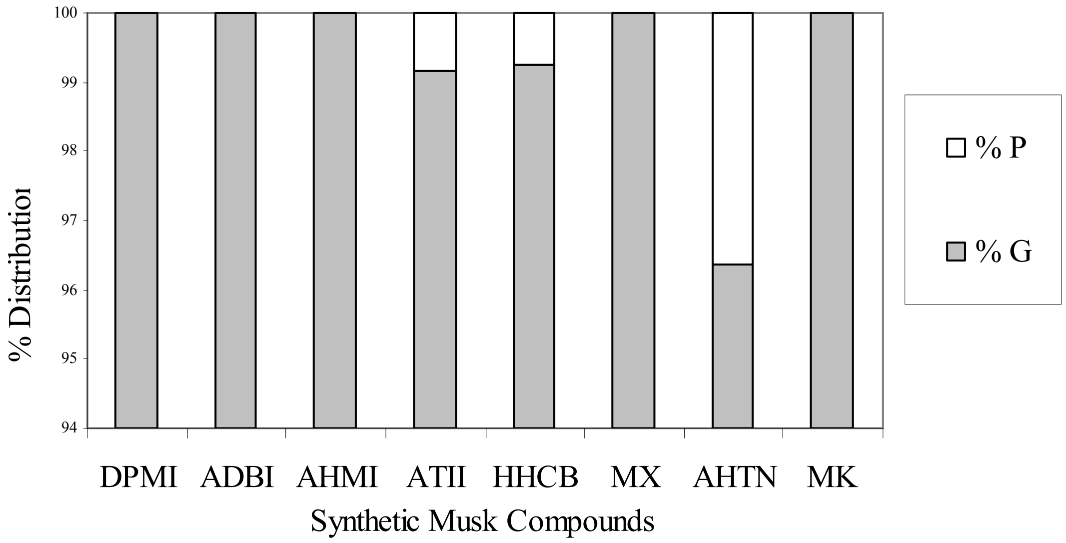


Figure 4.6. Gas and Particulate Phase Distribution in the Sport Center

Figure 4.7 illustrate the primary school samples gas phase distributions. It can be clearly seen that gas phase distributions were detected higher than particulate phase distributions. Five compounds, ADBI, HHCB, AHTN, ATII and MX have been determined on the particulate phase the others were 100 % on the gas phase at the classroom. These results similar to Chen et al. cosmetic plant study results where the musk compounds reported 86.35–97.70% in gas phase.

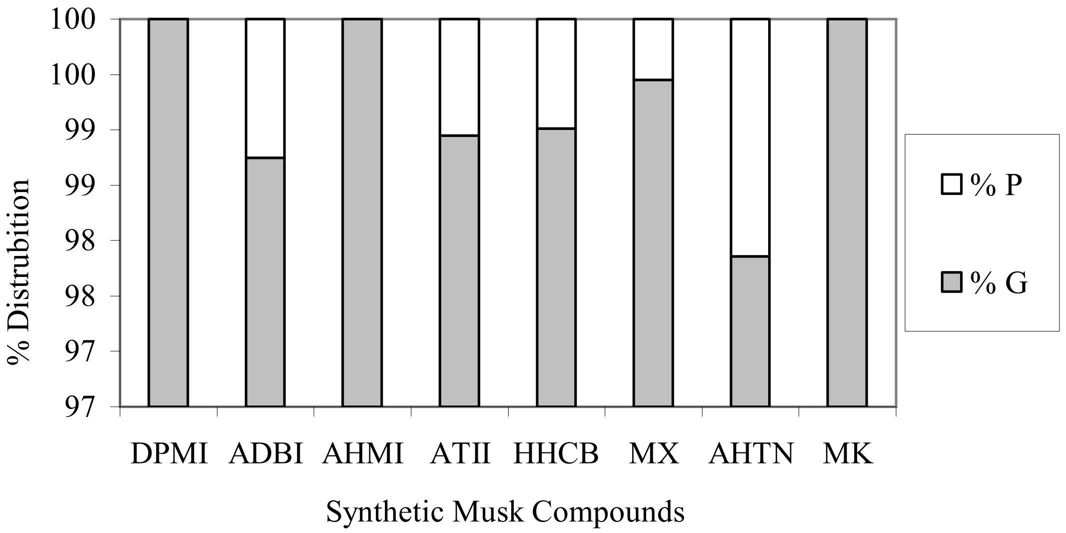


Figure 4.7. Gas and Particulate Phase Distribution in the Primary School

4.5. Risk Assessment

SCNNFP has conducted a human health risk assessment for oral exposure to synthetic musk compounds using toxicological data reported by Lehman-McKeeman et al. (1997) which characterized the profile and dose–response relationship of microsomal enzyme induction due to exposure to musk xylene. It was observed that liver weight and P-450 enzyme level were started to increase at doses >10 mg/kg and reached the maxima at 200 mg/kg with 65% increase in liver weight and two-fold microsomal cytochrome P-450 content compared to the control, in a dose-dependent manner.

SCCNFP concluded that if the oral absorption is 50%, no observed adverse effect level (NOAEL) may be assumed as 10 mg/kg/day. Calculations were based on lifetime cancer risk for the male mice for liver carcinomas or harderian gland tumors. The mice were dosed for 80 weeks, where they were dead after 90 weeks. After correlating the animal dose descriptor to the human dose descriptor, lifetime exposure dose representing a lifetime cancer risk of 10^{-4} was calculated as 7.3 µg/kg/day for both musk xylene and musk ketone.

The SCCNFP study was taken as guidance for the risk assessment in this study. In calculation of risk levels in the sport center and the primary school, 100% absorption for inhalation exposure route and heavy activity were assumed for a conservative approach. Exposure Factors Handbook (EPA, 1997) summarizes the recommended factor values for inhalation route. Recommended short-term heavy activity breathing rate for adults is 3.2 m^3/hr, and recommended short-term heavy activity breathing rate for children is 1.9 m^3/hr. The resulting doses were calculated for adults in the sports center and the children in the school based exposure durations of 1 hr/day and 8 hr/day, respectively. Average daily dose (ADD) is estimated with a general formula in equation 1 (EPA, 1997) which was mentioned in literature chapter and the risk estimates were calculated based on reported the lifetime cancer risk of 10^{-4} at 7.3 µg/kg /day. The calculated lifetime cancer risk levels are listed in Table 4.11. It was clearly seen that all estimates are well below the general acceptable risk level of 1×10^{-6}.

Table 4.3. Estimated Lifetime Cancer Risk for the Sport Center and the Primary School

Compound	Lifetime Cancer Risk 0f Urla Sport Center Samples	Lifetime Cancer Risk of Güzelyali Primary School Samples
DPMI	1.2×10^{-8}	6.2×10^{-8}
ADBI	7.4×10^{-10}	1.1×10^{-9}
AHMI	5.6×10^{-11}	1.3×10^{-10}
ATII	2.3×10^{-8}	4.3×10^{-8}
HHCB	1.1×10^{-7}	1.9×10^{-7}
MX	2.4×10^{-9}	7.2×10^{-9}
AHTN	2.9×10^{-8}	4.4×10^{-8}
MK	-	8.6×10^{-11}

CHAPTER 5

CONCLUSION

Eight synthetic musk compounds (6 polycyclic and 2 nitro musks) were measured for particulate phase and gas phase concentrations in the specified indoor air: primary school classroom and woman sport center.

The concentrations from high to low were ordered as HHCB, AHTN, and ATII for both sport center and classroom for particulate phase samples ($PM_{2.5}$). The values in primary school classroom were higher than sports center. All synthetic musk compounds were detected in primary school classroom but except musk ketone, the rest were found in the sport center gas phase samples. The descending order of the compounds for primary school classroom was HHCB, DPMI, AHTN, ATII, MX, ADBI, AHMI and MK. while it was like HHCB, AHTN, ATII, DPMI, MX, ADBI and AHMI for sport center. All of the synthetic musk compounds were dominated on the gas phase. The distributions between the phases were favor in gas phase and were above 95%.

Back-up samples showed that volatile compounds were collected carefully either shorter collecting time or some adsorbent resin should be used. The percentages were change between 76.74% to 88.65% for sport center and 86.24% to 96.67% for primary school samples were retained in the real samples media. The results were complied with available data in literature. Both polycyclic and nitro musks can be detected indoor air. They might be a pollutant for indoor air quality and affect the health. However if the SCCNFP risk estimations are taken into consideration calculated lifetime cancer risks very low when compared with a wide variety of oral risk estimations. However the use of synthetic musks as ingredients in household detergent and personal care products should be care with caution.

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