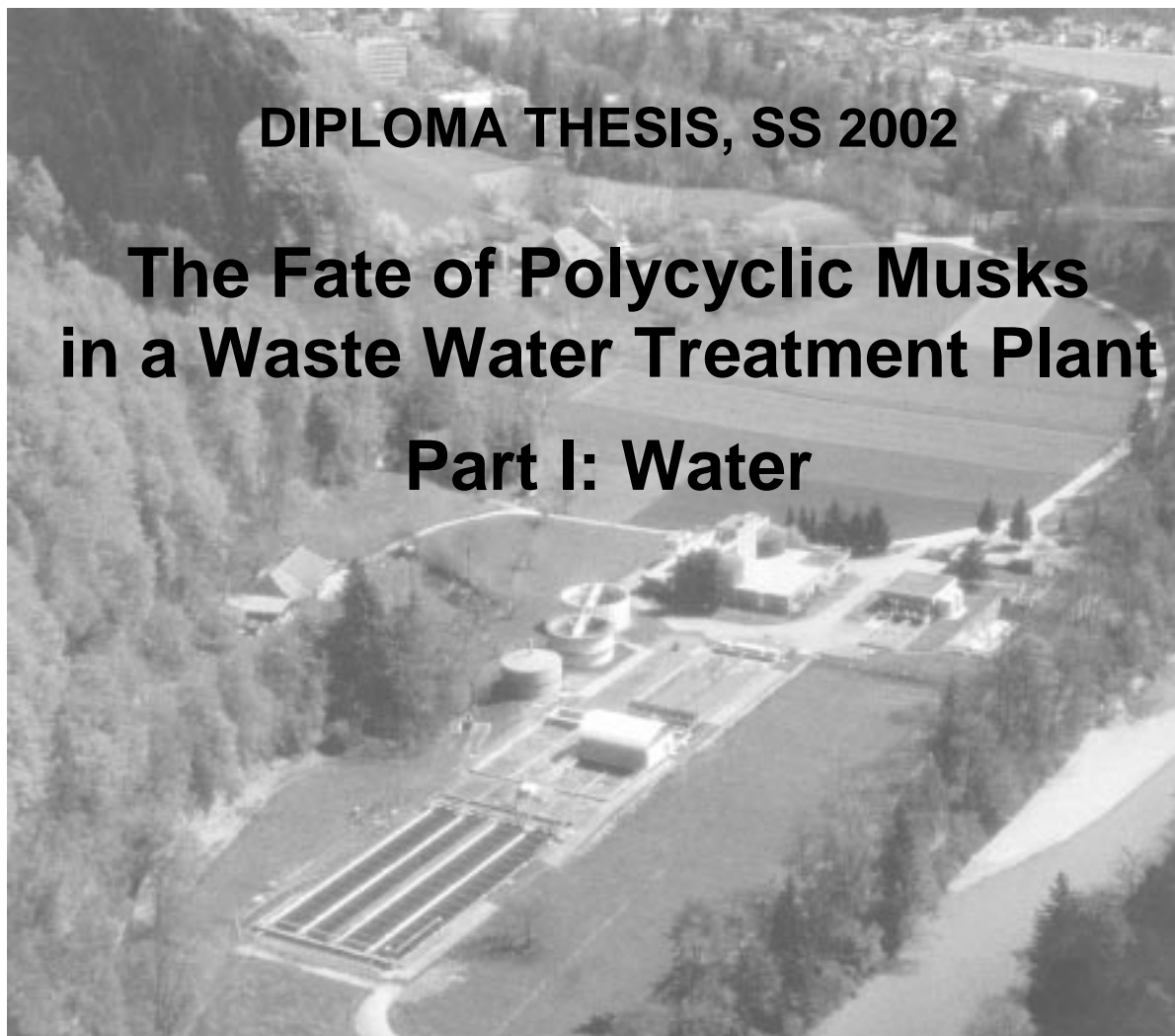


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# **The Fate of Polycyclic Musks in a Waste Water Treatment Plant**

## **Part I: Water**



Beobachtung des Stoffwechsels  
der Anthroposphäre im  
Einzugsgebiet ausgewählter  
Abwasserreinigungsanlagen

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## Abstract

A method to analyse polycyclic musks in waste water samples by detection in the GC-MS has been established. During a week, concentrations of these lipophilic compounds and of one of their metabolites have been monitored in water samples taken from a representative waste water treatment plant in Switzerland. Galaxolide and Tonalide concentrations measured in waste water were 4500 ng/l and 1450 ng/l respectively. Other polycyclic musk concentrations in waste water did not exceeded 180 ng/l. In treated waste water, Galaxolide and Tonalide concentrations were 780 ng/l and 330 ng/l respectively, whereas concentrations of the other polycyclic musk compounds were lower than 30 ng/l.

It was found that polycyclic musk concentrations decrease significantly during the waste water treatment process, while the metabolite's concentration remained stable. Transfer coefficients in primary settling, biological treatment and in the entire waste water treatment process have been computed. For Galaxolide and Tonalide they are 17% and 21% in primary treatment and 81% and 77% for the entire waste water treatment process, which is in accordance with values found in recent studies. No correlation could be established between the concentrations of the different compounds, indicating that dissimilar mixtures are used as fragrances.

In order to assess the fate of polycyclic musks in the waste water treatment plant, a material flux analysis is established, accounting for all fluxes. Polycyclic musk concentrations in sludge are calculated thereof and are compared with values measured in other studies and values obtained by other calculations. Results obtained will be verified by sludge analysis in a second phase of the project.

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# 1. Introduction

## 1.1 Production and Consumption of Polycyclic Musks

Synthetic musk ingredients are used widely in most fragrances for detergents, fabric softener, fabric conditioners, cleaning agents, air fresheners and cosmetic products such as soap, shampoos and perfumes (OSPAR Commission, 2000). Three groups of chemically very different musk compounds are produced: nitro musks, polycyclic musks, and macrocyclic musks (for chemical structures see Appendix 1.1). However, all three of them have the same musky smell, which is characteristic for closed-packed molecules with a molecular weight between 200 and 300 and characteristic arranged functional groups (Liu et al., 1998). It is interesting to see that from the about 1000 known compounds with a musky smell (Rebmann et al., 1997) only a few have commercial importance.

Originally musk was harvested from musk deers (Moshidae). These are goat sized cloven hoofed animals, that are characterized by their abdominal musk gland (Myers, 1999). This gland is found by males only and secretes a brownish, waxy substance, called musk, which smells the strongest during mating season. In the Koran and in thousands of years old perish scripts musks are already mentioned as a valuable perfume and in the occidental world the history of musk goes back to antiquity (Wiegel et al., 2000). In order to harvest musk, the musk deers have to be killed. This may be the main reason why musk deers are listed in the CITES-Database on Appendix I or II, depending on the species and the country (UNEP-WCMC, 2002). Species recorded on the CITES Appendix I are considered as being threatened with extinction and therefore trade in specimens of these species is permitted only in exceptional circumstances, whereas species in Appendix II are not necessarily threatened with extinction but may become so unless trade is closely controlled.

Natural musk, belonging to the group of macrocyclic musks, is still very valuable. The actual state in 1999 was 50'000 euros per kilogram as outlined by Wiegel et al. (2000). Nowadays most of the musks are synthesised chemically. In addition to their typical and unique smell, musks are added to products as well due to their ability to improve the quality of other fragrances. They make them more balanced and longer lasting, because of their ability to bind fragrances to fabrics and to the skin (OSPAR, 2000). The first artificial musks, nitro musks were synthesized by coincidence towards the end of the 19<sup>th</sup> century while searching for organic explosives as reported by Wiegel et al. (2000). Besides the use as fragrances, nitro musks were added in commercial herbicide formulations as reported by Yamagishi (1983). Since no other evidence of this use could be found, it is assumed that nitro musks were added to herbicides in Japan only.

Polycyclic musks, the other important group of synthetic musks, were introduced in the 1950s (Rimkus, 1999). They have the ability to make fragrances longer lasting and to balance the initial and the residual smell (Ford, 1998), the main reason why they are highly recognized by the fragrance industry. Macrocyclic musks are highly esteemed by the perfume industry because their ability to fix the smell of perfume to skin is much higher than in other synthetic musks (Rebmann et al., 1997). However their synthesis is complex and therefore they are rather expensive, which explains why macrocyclic musks are used mostly in perfumes.

Rebmann (1997) states that research for musk compounds is ongoing and that every year new fragrances are patented. It can be assumed that the newly patented musks exhibit the same chemical properties, since they are designed to perform in the same way. Therefore it is concluded that the new musks may act as environmental contaminants as nitro and polycyclic musks. Nitro- and polycyclic musks do not occur in nature and due to their specific use and their low biodegradability, they are found in waste water at high concentrations. Polycyclic musks are therefore suitable indicator to monitor contamination of drinking water by waste water as Noser et al. (1999) and Fromme et al. (2000) assessed.

In 1987 the worldwide production of musks was 7000 tonnes with a commercial value of 215 Mio US\$ (Rebmann et al., 1997). Polycyclic musk compounds exhibit by far the highest production volume (Table 1.1 and Table 1.2). Bridges (2002) reports that nitro musks are used less in Europe than in the US and that Europe has a leading position in regulating the use of

musks. It is difficult to account for production rates, since they are usually cited from inter sources and then recited through the literature.

Table 1.1: Use of nitro musks in Europe (in tonnes) (for trade and chemical names, see Appendix 1.2)

Year	Musk xylene (t)	Musk ketone (t)	Muskene (t)	Musk tibetene (t)
1992 <sup>1</sup>	174	124		
1995 <sup>1</sup>	110	61	5	0.8
1998 <sup>1</sup>	86	40		
2000 <sup>2</sup>	67	35.3		

Table 1.2: Use of polycyclic musks in Europe (in tonnes) (for trade and chemical names, see Appendix 1.3)

Year	Galaxolide (t)	Tonalide (t)	Celestolide (t)	Phantolide (t)	Traseolide (t)
1992 <sup>1</sup>	2400	885			
1995 <sup>1</sup>	1482	585	34	50	40
1998 <sup>1</sup>	1473	385	18	19	2
2000 <sup>2</sup>	1427	343			
Europe and US <sup>3</sup>	3800	1500			
worldwide 1996 <sup>4</sup>	polycyclic musk total 5600				

<sup>1</sup> Source: (OSPAR Commission, 2000)

<sup>2</sup> Source: A. Weber (SAFEL), Personal communication, source IFRA

<sup>3</sup> Source: (Ford, 1998)

<sup>4</sup> Source: Gebauer and Bouter (1997) cited in (Rimkus, 1999)

The quantity of nitro musks used in Europe is declining over the last years. This is not surprising since the EU banned the use of musk tibetene and muskene in 1998 by including these substances in the list of banned components in cosmetics (98/62/EC of 3rd September 1998), because they were found in the environment. For musk xylene and musk ketone limit concentrations for several cosmetic products were introduced (Commission Decision 2002/34/EC of 15<sup>th</sup> April 2002). These maximum authorised concentrations in the finished cosmetic product for musk xylene are 1% in fine fragrance, 0.4 % in eau de toilette and 0.03% in other products. Values for musk ketone are 1.4%, 0.56% and 0.042% respectively. In Germany (1993) and Switzerland (1994), the industry association responsible for detergents recommended to their members companies not to use musk xylene any more in washing and cleaning agents (OSPAR Commission, 2000). The International Fragrance Association (IFRA) recommended to their members 1995 to abandon the production of nitro musks (Rebmann et al., 1997). Rebmann explains that in China only nitro musks are still produced on a quite large scale (800-900 tons/year). The EU has also set criteria for eco-labelling hand dishwashing detergents and among other criteria; the products must not contain nitro musks or polycyclic musks. (Commission Decision 2001/607/EC, 19th July 2001)

Due to the actions already taken and the general concern about the environmental fate and toxicology of nitro musks, their importance as fragrances is decreasing (OSPAR Commission, 2000). It is interesting to see that even though nitro musks are not as stable in light and olfactory inferior to polycyclic musks (Rebmann et al., 1997), it took severe measures in order to lower their use. One of the reasons may be the lower production costs of nitro musks. The OSPAR Commission (2000) was concerned that the ban of nitro musks may lead to an increased production of polycyclic musks, but as the use of polycyclic musks shows Table 1.2, this concern cannot be confirmed. Nevertheless, the polycyclic musks Galaxolide and Tonalide are high volume chemicals in Europe (Rimkus, 1999) and therefore an environmental risk assessment has been carried out for these compounds (Van de Plassche et al., 1997). A summary of it was published 1999 in Toxicology Letters (Balk et al., 1999b; Balk et al., 1999a).

Due to the decline of the use of nitro musks the following report will focus on polycyclic musks only. The compounds considered are: Galaxolide (HHBC), Tonalide (AHTN), Celestolide (ADB1), Phantolide (AHMI), Traseolide (AITI), Cashmeran (DPMI) and a metabolite of Galaxolide, Galaxolidone. The chemical structures and properties of all the compounds analysed are described in Appendix 1.4. Versalide (ATTN or AETT) is not included since its production was already discontinued in 1980 because of the evidence of strong adverse neurotoxic effects (Rimkus, 1999).

## 1.2 Environmental Exposure

Environmental contamination by musk xylol and musk ketone, two nitro musks was detected for the first time in fresh water fish from the Tama River in Tokyo (Yamagishi et al., 1981). Yamagishi et al. (1983) suggested in their second publication about nitro musks that musk xylene and musk ketone exist as bioaccumulation type pollutant in the aquatic or marine environment. Consequently the use of nitro musk compounds was banned in Japan in the early 1980s (Bridge, 2002). 1983 musk xylol was found in one of three fish samples taken from a lake in Illinois (US) (Yurawecz et al., 1983). It is interesting to see that this finding was interpreted as a contamination of the sample outside the aquatic environment and no further investigations were made.

The first reports about environmental contamination by nitro musks in Europe date from 1993. Nitro musks were found in European trouts during food monitoring (Rimkus et al., 1993a). It was searched for organochlorine pesticides, PCBs and mercury originally, when two intensive, not identifiable peaks were found in the GC/ECD chromatogram. One of them was identified by Rimkus as musk xylol. The publication of this identification by GC/MS could not be found, since the article referred to in Rimkus et al. (1993a) refers back to the original article (Rimkus et al., 1993c). Rimkus reports for the first time the detection of five nitro musks (musk xylol, musk ketone, musk ambrette, musk mosken, musk tibetene) in trouts from aquaculture, fish and mussels from German surface waters (Rimkus et al., 1993a). Since nitro musks in fish were considered to harm human, sources and contamination in human were investigated. Hahn found 1993 that the main source was not as assumed fish food that was enriched with musk xylol in order to cover the displeasing odour of the fish feed but sewage, that contained fragrances from detergents. Rimkus analysed nitro musks in human milk, which is widely used to identify and monitor body burden of lipophilic persistent environmental pollutants and human fat. Musk xylol and musk ketone were found in all his samples (Rimkus et al., 1993b). These results were confirmed by others (Rimkus et al., 1994; Liebl et al., 1993). Consequently Geyer et al. (1994) asked the fragrance industry to abandon the use of nitro musk for the precautionary principle. As described before the EU banned the use of musk tibetene and muskene in cosmetic products in 1998 and introduced limit concentrations for several cosmetic products for the two most important nitro musks, musk xylene and ketone in 2002.

Galaxolide, Tonalide and Celestolide, three polycyclic musk compounds were analysed for the first time in surface water, waste water and fish samples in 1994 (Eschke et al., 1994). Mean polycyclic musk concentrations in sewage were measured 1.46 µg/l for Galaxolide, 2.24 µg/l for Tonalide and 0.08 µg/l for Celestolide. Waste water treatment plant effluent concentrations were at 1.09 µg/l for Galaxolide, 1.4 µg/l for Tonalide and 0.06 µg/l for Celestolide. During a non-target screening of water samples taken between 1992 and 1994 from the River Elbe and its tributaries, more than 250 individual organic compounds were identified, among them Galaxolide and Tonalide (Franke et al., 1995). Phantolide and Traseolide were identified 1995 in waste water, surface water and fish samples, whereas Cashmeran and Versalide, which production was abandoned long time ago could not be found (Eschke et al., 1995).

Polycyclic musks were found for the first time in human milk and human fat in 1995 (Eschke et al., 1995). There were only two fat and two milk samples analysed in this study, which makes it impossible to draw a valid conclusion about the burden in humans from this publication. However, it is interesting to see that besides Galaxolide (147 ng/g fat in fat and 335 ng/g fat in milk) and Tonalide (64 ng/g fat in fat and 270 ng/g fat in milk), even though at much lower concentration, Celestolide, Phantolide and Traseolide had been detected also, whereas DPMI was not found. The route of exposure by oral uptake by consumption of fatty food, does not



seem to be decisive in the case of musk compounds (Liebl et al., 2000). Rimkus (1997) stated that that percutaneous absorption seemed to be much more relevant as cited by Liebl et al. (2000). This seems to be logical because as reported before musks are used to improve the binding of fragrances to skin. However margins of safety based on the no-adverse-effect levels from subchronic studies are sufficient to conclude that direct exposure from the use of Tonalide and Galaxolide in fragrances or indirect exposure from their presence in the environment pose no significant risk to human health (Ford, 1998). Even there seems to be no risk for humans, environmental threatening should not be underestimated. In many studies polycyclic musks have been analysed in water, waste water, surface water and sewage sludge. A summary of all the analyses conducted is given by Rimkus (1999) and a summary of the concentrations found in waste water, treated waste water and in sludge is given in Appendix 1.5 and 1.6.

Polycyclic musk compounds were not found in waste water samples only, even though they have low  $K_H$  values, a Norwegian study found synthetic musks in outdoor air, not only in urban areas but also in remote areas (Kallenborn et al., 1999). Rate constants for the gas reaction with OH radicals have been measured for Galaxolide and the atmospheric life time for this compound was concluded 5.3 h (Aschmann et al., 2001). It can be deduced that this compound is not transported over long distances by air.

As can be seen, almost all investigations were made in Europe. In Canada for the first time nitro and polycyclic musks were determined in aquatic fauna in 1999 (Gatermann et al., 1999). Since nitro musk concentrations exceeded polycyclic musk concentrations, the results seem to reflect the different application modes in Western Europe and North America as claimed by the authors. On the other side Simonich et al. (2002) do not find significant different concentrations (on a 1% level) in waste water treatment plant influent for Galaxolide and Tonalide in the US and Europe.

The contamination of the environment and the occurrence of musks in human tissue and human milk is not surprising if their significant lipophilic properties (see  $K_{ow}$  values, Appendix 1.4), their low biodegradation rates (biodegradation rate is set to  $0 \text{ hr}^{-1}$  in the environmental risk assessment by van de Plassche et al. (1997) and their considerable production rates are considered

### 1.3 Toxic and Ecotoxic Effects

Toxicity data for a few polycyclic musk compounds were determined for some aquatic and terrestrial organisms (see Appendix 1.7). Since, as explained above, this report will focus on polycyclic musks, data for other musk compounds were not collected. Concentrations found in treated waste water, which is the most contaminated aqueous matrix, are still much lower than the predicted no effect concentration (PNEC) for water organisms (Appendix 1.7). This means that there is no threat by toxicological effects on the animals assessed yet. For soil organisms and predators no conclusion can be drawn since in these compartments musk concentrations have not been measured yet. However systemic toxicity is not the only effect of chemicals.

Versalide (ATTN or AETT) was found to be neurotoxic whereas for Galaxolide, Tonalide and Celestolide no neurotoxic effects have been found up to now (Wiegel et al., 2000). Galaxolide and Tonalide have a weak estrogenic but no uterotrophic potency. However, the estrogenic effect is too weak to induce estrogenic effects in wildlife species or humans at the current levels of exposure as explained by Seinen et al. (1999). Tonalide and Galaxolide induced teratogenic effects in larvae of zebrafish (*Danio rerio*) (Schreurs et al., 2001).

As published by Ford (1998) Galaxolide and Tonalide are no skin irritants and show no potential for sensitization in human and animal screening studies. A combination of low systemic toxicity from subchronic studies and the total lack of genotoxicity indicates no potential for long term effects (Ford, 1998). Therefore Ford concludes that the presence of Galaxolide and Tonalide do not pose a significant risk to human health from direct as from indirect exposure. However Wiegel (2000) argues that cancerogenity needs to be assessed further.

Bioconcentration is defined as the direct uptake of a chemical by an organism only from water and is quantified by the bioconcentration factor (Rimkus, 1999). Bioconcentration factors on a wet weight are around 620 and 560 for Galaxolide and Tonalide respectively (Geyer et al., 2000) (see also Appendix 1.7).

However Gatermann et al. (2000) found that bioaccumulation factors of Galaxolide and Tonalide in fish vary up to a factor 20 and they concluded that metabolism of musks is species dependent and therefore caution has to be applied if risk assessment studies have to rely on one fish species only.

#### **1.4 Project SEA (Observation of the metabolism of the anthroposphere)**

As can be seen musks, especially polycyclic musks have not been an issue for a long time but they are still a subject of concern as can be noticed by recent publications in the consumer's magazine Saldo (1/02) and Ökotest ([www.ökotest.de](http://www.ökotest.de)) and activities of OSPAR commission. Due to their chemical properties namely their lipophilicity and their toxicity, they pose a potential threat to the environment and consequently to human. Therefore a close monitoring is essential. Waste water treatment plants are named the sources of environmental contamination by polycyclic musks because, due to the use of musks as fragrances in detergents and cosmetic products, it can be assumed that most of these compounds end up in waste water treatment plants influents.

This research project is written within the frame of the project SEA. The project describes contaminants and their sources in the anthroposphere, as well as their distribution in the environment. Relevant substances are analysed in sewage sludge sampled in waste water treatment plants of a monitoring network. Sewage sludge contains nearly all important compounds of the anthroposphere, which makes it like the mirror of our society. Based on the methodology of the material flux analysis, the analytical results are evaluated. The aim of the SEA project is to detect pollutants early and to contribute to the elaboration of measures reducing impacts on the environment.

For modelling the sources of pollutants and their distribution in the environment based on sewage sludge, the properties of the wastewater treatment plant (WWTP) and its catchment's area must be known. This includes the technology of wastewater treatment, the collector system, the socio-economic structure as well as activities of industry and craft industry in the catchment's area. Therefore a monitoring network constituted of about 30 monitoring sites (WWTP and its catchment's area) in some Swiss cantons (VD, FR, BE, LU, BL, AG and ZH) was set up. This allows linking the presence of the compounds in sewage sludge with the characteristics of clearly defined catchment's areas, which is helpful to draw conclusions from the analytical results. This monitoring network contains three types of observation sites: Type A (10 sites): rural catchment's area, no industry and very few craft industry in the catchment's area, separate sewer system (domestic waste water only), Type B (15 sites): rural catchment's area, no industry and very few crafts industry in the catchment's area, combined sewer system (domestic waste water and runoff water) and Type C (9 sites): urban catchment's area, industry and craft industry in the catchment's area, combined sewer system (domestic waste water and runoff water). These three types of waste water treatment plants are the base for the assessment of the pollutants from the three main sources: private households, industry and atmospheric deposition.

This research project focuses at the fate of polycyclic musks in a typical waste water treatment plant in Switzerland. Since the aim of the SEA project is to conclude from polycyclic musk concentrations in sludge to influent and effluent concentration and the use of polycyclic musks, it is necessary to compute transfer coefficients between water and sludge, hence to measure polycyclic musk concentrations in influent and effluent, which is accomplished in this study.

## 2. Objectives

Fragrances added to detergents, fabric softeners and cosmetics are discharged after use to domestic waste water and find their way to the sewage treatment plant. In order to assess the behaviour of polycyclic musks, an important fragrance compound in the environment, it is essential to examine the fate of these substances in the waste water treatment plant, the main origin of environmental contamination by polycyclic musks. An appropriate mean is a material flow analysis, as it was examined for heavy metals in a waste water treatment plant by Goldstone (Goldstone et al., 1990b; Goldstone et al., 1990c; Goldstone et al., 1990a; Goldstone et al., 1991b; Goldstone et al., 1991a). This knowledge allows to assess whether musks are degraded during the waste water treatment process and to know if they accumulate in sludge or if they are released via effluents. Results will contribute to track main sources of environmental contamination by polycyclic musks and play a role in the discussion on safety of sludge application to agricultural land. From the knowledge of the transfer coefficients established in a material flow analysis, it is possible to extrapolate from sludge analysis the flows in waste water and treated waste water.

In order to establish a material flow analysis polycyclic musks have to be analysed in water as in sludge at different stages of an appropriated waste water treatment process. To account for daily variations concentrations should be measured over a certain time and ideally more than once during a year. The first objective of this project was therefore to find an appropriated method to analyse polycyclic musks in water and sludge within the possibilities of the Laboratory for Environmental Chemistry and Ecotoxicology (CECOTOX) at the Swiss Federal Institute for Technology in Lausanne. Unfortunately it was not possible to establish a method to analyse sewage sludge fully within the time limits of this project. Therefore it is focused on the water analysis method only. In a second phase of this project different water samples taken from a representative waste water treatment plant are analysed and variation during the sampling week are monitored. Concentrations of polycyclic musks in sludge are estimated by balancing the masses in the waste water treatment plant and by estimating concentrations by transfer coefficients already determined in the literature and the  $K_{oc}$  values. Results found in other SEA studies are compared with the results computed. Polycyclic musk concentrations in sludge, which is already sampled, will be analysed later, allowing to complete the material flow analysis and to assess whether degradation within the plant is substantial. A complete material flow analysis will admit to extrapolate from sludge analysis to polycyclic musk concentrations in waste water and treated waste water and to examine the uncertainties in the risk assessment published by Balk (1997), which was not possible up to now due to the lack of data as explained by Schwarz et al. (2000).

Due to the decline in the use of nitro musks, this study focuses on polycyclic musks only. The musk compounds considered are Cashmeran (DPMI), Celestolide (ADBI), Phantolide (AHMI), Traseolide (AITI), Galaxolide (HHCB), Tonalide (AHTN) and a metabolite of Galaxolide, Galaxolidone. The analysis of a metabolite allows accounting for the degradation rate of Galaxolide and since all these compounds have already been analysed in other SEA projects, results can be compared. Chemical structures and properties of the polycyclic musks analysed are listed in Appendix 1.3 and 1.4.

## 3. Sampling and Analytical Method

### 3.1 Waste Water Treatment Plant

All samples were taken from the waste water treatment plant 'Mittleres Emmental' in Hasle (BE), Switzerland, (for location see map in Appendix 3.1) which belongs to the SEA monitoring network. The site 'Mittleres Emmental' is classified as a Type C site (Kupper et al., 1999) meaning that the catchment's area has a unitary sewer system and that there are industry and craft industry in the 17 mainly rural communities connected to the waste water treatment plant (map of the catchment's area in Appendix 3.3). The treatment plant receives sewage originating from households, industry, trade and from run-offs. It was chosen for this research project due to its representativity for a Swiss sewage treatment plant referring to the catchment's area and the waste water treatment method and because it is technically up to date. Furthermore flow rates are measured in detail and the different tanks and sampling places are accessible easily. The waste water treatment plant 'Mittleres Emmental' serves a population of 22716, receiving about 9300m<sup>3</sup> of sewage a day at dry weather and about the double if it rains. The average pollution load is 1100 kg/d (BOD<sub>5</sub>) (Personal communication: B. Bangerter, GSA Berne). Waste water entering the plant is first treated mechanically using a gravel trap, a screen (16mm) and a sand trap to remove the larger solids (schematic overview of the plant in Appendix 3.2). Waste water is then passed to the primary settling tank where additional solids are removed by gravitation as primary sludge. After two hours water is moved to the activated sludge system consisting of four parallel reactors. Precipitant (FeClSO<sub>4</sub>) is added to the aerated tank Picture 3.1 (simultaneous precipitation).

In the secondary settling tank activated sludge settles and after approximately two hours treated waste water is released to the receiving water course, Emme. Most of the activated sludge settled in the secondary settling tank is returned to the biological treatment tanks, whereas a small amount (1/85) is pumped to the primary settling tank, where it settles with primary sludge. Primary sludge is moved to the thickener and is allowed to settle and thicken for a day. Supernatants are then decanted and pumped to the head of the plant while the thickened sludge is moved to the disinfection reactor. It is disinfected for one hour at approximately 60°C. Digestion of the about 100 m<sup>3</sup>/d (4% dm) primary sludge results in 40 m<sup>3</sup>/d (8% dm.) digested sludge and 1200 m<sup>3</sup>/d gas. Gas is used as an energy source within the plant and sludge is stored in the sludge storage tank until it is spread on agricultural land or brought to incineration.



Picture 3.1

The waste water treatment plant 'Mittleres Emmental' reduces 96% of influent biological oxygen demand (BOD<sub>5</sub>), 90% of the chemical oxygen demand (COD), 24% of total nitrogen, 87% of ammonia nitrogen and 86% of total phosphorous (personal communication: B. Bangerter).

### 3.2 Sampling

#### 3.2.1 Flow Rates

Flow rates were measured at the locations indicated in Figure 3.1. Waste water influent was computed continuously and every fifteen minutes an average inflow rate (l/s) was reported. The quantity of waste water entering the plant between 7.30 and 7.30 of the following day was calculated from these data according to the sampling period. The amount of supernatants from

the storage tank and sludge quantities was taken as reported by the waste water treatment plant. Flows ( $\text{m}^3/\text{day}$ ) measured during the sampling period are reproduced in Table 3.1. From these flows remaining flows in the treatment plant were computed, assuming that the quantity removed from the waste water by screening and the gravel trap is negligible little ( $26\text{m}^3$  in April 2002) and that there are no losses within the plant. Results are summarised in Table 3.1

Table 3.1: Measured and calculated flow rates ( $\text{m}^3/\text{day}$ ) in the waste water treatment plant 'Mittleres Emmental' during the sampling period

		9.04.02 Tues	10.04.02 Wed	11.04.02 Thurs	12.04.02 Fri	13.04.02 Sat	14.04.02 Sun	21.04.02 Sun	21.04.02 Sun	22.04.02 Mon	23.04.02 Tues
inflow wwtp (7.30-7.30)	m	7'904	7'784	7'689	7'986	9'623			7'035	7'783	
influent primary settling tank	c	8'125	8'011	7'925	8'165	9'766			7'169	8'002	
influent biological treatment tank	c	8'026	7'915	7'830	8'066	9'674			7'080	7'922	
influent secondary settling tank	c	18'436	18'345	18'180	18'356	24'104			15'800	18'082	
effluent	c	7'906	7'795	7'710	7'946	9'554			6'960	7'802	
Difference effluent-influent (%)	c	0%	0%	0%	1%	1%			1%	0%	
activated sludge	c	10'530	10'550	10'470	10'410	14'550			8'840	10'280	
sludge returned	m	10'410	10'430	10'350	10'290	14'430			8'720	10'160	
excess sludge	m	120	120	120	120	120			120	120	
primary sludge	m	99	96	95	99	92	82		89	80	94
supernatants from thickener	c	11	17	26	32	14	14		14	1	14
sludge to disinfection	m	88	79	69	67	78	68		75	79	80
supernatants from sludge storage tank	m	90	90	90	27	9	0	87	0	98	100

wwtp: waste water treatment plant, m: measured, c: calculated

The weather was dry during almost the whole sampling period. Saturday evening 13.4.2002, there was some heavy rain; however the sampler sampling the waste water broke down shortly after the rain water had reached the treatment plant. Inflow rate for this day was calculated up to the time the sampler stopped and then extrapolated to one day. This results in a lower inflow rate as actually measured by the waste water treatment plant, but since inflow rates to the biological treatment and effluent rates are not measured but calculated from inflow rates no difference results from this extrapolation. Inflow and calculated outflow rate do not differ substantially on the other days neither as can be seen from Table 3.1.

There were spring holidays in most of the public schools in the catchment's area during the first week of the sampling period with the back to school day on the 22<sup>nd</sup> of April. This might have influenced flow rates and musk concentrations due to other washing activities during this time. However considering the inflow rates of the entire month, this hypothesis cannot be confirmed, since they are neither lower nor higher the days of school holidays.

### 3.2.2 Sampling Methods

Within the waste water treatment plant samples were taken at the influent, after primary settling, at the effluent, from supernatants and different types of sludge during one week from 9<sup>th</sup> to 14<sup>th</sup> April 2002. Unfortunately the sampler sampling the waste water entering the plant stopped working several times during Sunday, 14.04.2002 and the following two days. Therefore sampling was repeated at the beginning of the following week in order to cover a sampling period of one week. Sampling locations are represented in Figure 3.1. Dark arrows indicate that daily samples were analysed, grey arrows represent that samples were joined before extraction in order to reduce the number of analysis required and light arrows identify discrete samples

taken once during the sampling week. Approximate retention times in the tanks are indicated in brackets. The sampling plan including sample numbers is represented in Figure 3.2.

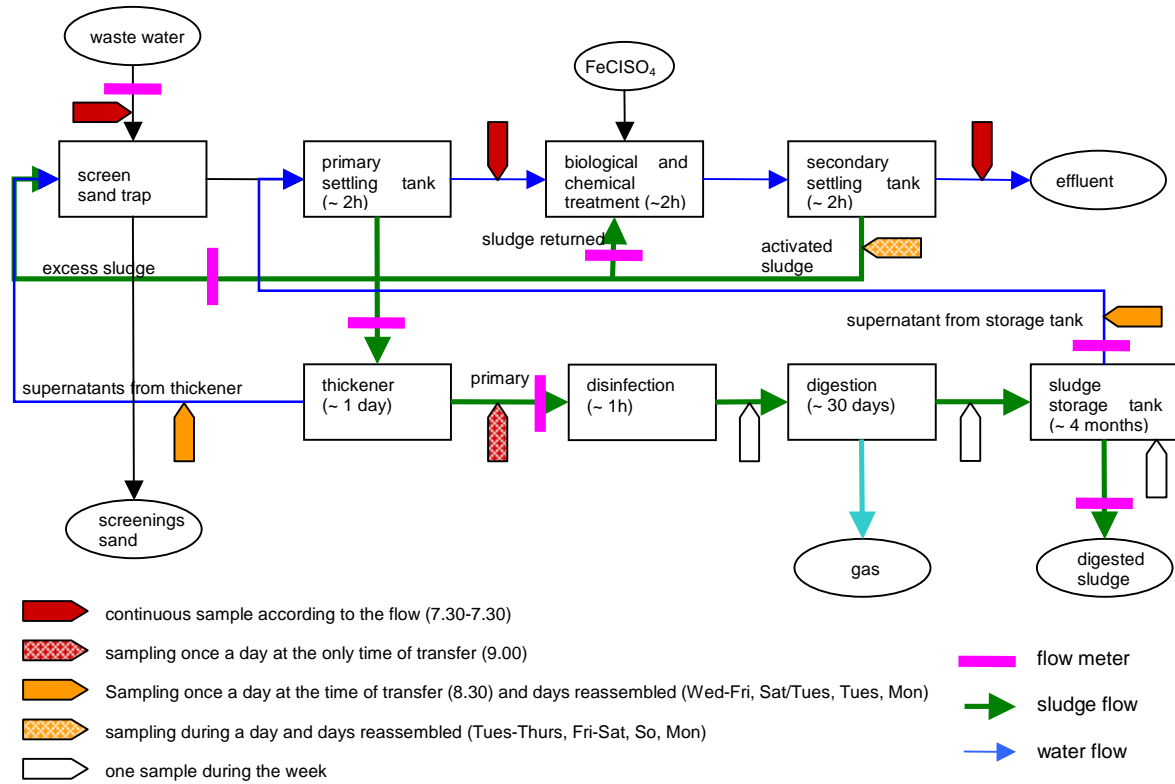


Figure 3.1

Schematic overview of the waste water treatment plant 'Mittleres Emmental', indicating sampling location and flow meter

	Tuesday 09.4.02	Wednesday 10.4.02	Thursday 11.04.02	Friday 12.04.02	Saturday 13.04.02	Sunday 14.04.02	Mon 15.04	Tuesday 16.04.02	Monday 22.04.02	Tuesday 23.04.02	
waste water		X 1 sample no. 269	X 1 sample no. 276	X 1 sample no. 285	X 1 sample no. 292	X 1 sample no. 299	Sampler did not work	Sampler did not work	X 1 sample no. 307	X 1 sample no. 314	
water after primary sedimentation		X 1 sample no. 270	X 1 sample no. 277	X 1 sample no. 286	X 1 sample no. 293	X 1 sample no. 300			X 1 sample no. 308	X 1 sample no. 315	
treated waste water		X 1 sample no. 271	X 1 sample no. 278	X 1 sample no. 287	X 1 sample no. 294	X 1 sample no. 301			X 1 sample no. 309	X 1 sample no. 316	
primary sludge		X 1 sample no. 272	X 1 sample no. 279	X 1 sample no. 288	X 1 sample no. 295	X 1 sample no. 302			X 1 sample no. 310	X 1 sample no. 317	
activated sludge returned		X ½ sample No. 274	X ½ sample no. 281	X ½ sample no. 290	X ½ sample no. 297	X ½ sample no. 304			X 1 sample no. 312	X 1 sample no. 319	
supernatants from thickener		X ½ sample no. 273	X ½ sample no. 280	X ½ sample no. 289	X ½ sample no. 296	X ½ sample no. 303			X 1 sample no. 311	X 1 sample no. 318	
supernatants from the sludge storage tank		X ½ sample no. 275	X ½ sample no. 282	X ½ sample no. 291	X ½ sample no. 298	was not removed on a Sunday			X 1 sample no. 305	X 1 sample no. 313	X ½ sample no. 320
sludge after disinfection			X 1 sample no. 283								
sludge from digester									X 1 sample no. 306		
sludge from stock			X 1 sample no. 281								

X = collection of the samples (in the morning at 7.30)

-- = continuous sampling

Figure 3.2

Sampling plan, including sampling numbers

In the following paragraph sampling methods for all samples taken are described even though water samples only were analysed in this first part of the project.

### Waste Water

A flow related sampler (Type: Bühler PB-MOS) was installed after the first bend following the gravel trap (see Appendix 3.2). A sample of 50-85 ml was taken every time 30-50 m<sup>3</sup> water had flown by the sampling station. The sampler stopped working several times as charging device of the battery did not run properly. In order to avoid interruptions sampling frequency was diminished and the quantity of the aliquot was slightly increased at flow rates above 110 l/s. Sampling operations are reported in detail in Appendix 3.4. Samples were collected in a glass bottle placed in a refrigerator to ensure storing in the dark at a temperature of about 4°C (Picture 3.2).

A discrete waste water sample was taken the last day, of the sampling period in order to test the analysis method.



Picture 3.2



Picture 3.3

### Waste Water after Primary Settling and Treated Waste Water

Water after primary settling as treated waste water were sampled with a flow related sampler of Type: IBUK-KS 350, IBUK PNV-MC, which is operated by the sewage treatment plant for daily sampling. A sample of 75 ml was taken every 20 m<sup>3</sup>. The samples were collected in a glass bottle, placed in a metallic box to ensure storing in the dark at a temperature of 4°C (Picture 3.3).

Bottles of the continuous sampling stations were exchanged every morning at about 7.30. This operation allowed a good distinction of sampling days as human activities start about 6.00 am and the main water flow reaches the treatment plant with a delay of about two hours due to the extended catchment's area.

### Supernatants from the Thickener

Primary sludge is settled in the thickener in order to separate liquids from sludge. Before primary sludge is transferred to the disinfection tank, supernatants are pumped from the thickener to the head of the sewage treatment plant. Samples were taken about every 10 minutes during the only time of transfer in the morning. They were collected in a glass bottle, which was protected from sunlight.

### Sludge from the Thickener

After supernatants from the thickener had been transferred, sludge was mixed for 25 minutes approximately. Then samples were taken by lowering a little metallic bucket, attached to a string into the thickener (Picture 3.4). These aliquot samples were collected in an aluminium container.



Picture 3.4



Picture 3.5

### Activated Sludge

Aliquot samples of activated sludge were taken several times during the day from the transfer pipe of return sludge. They were collected in an aluminium container. Exact sampling times and quantities are reported in Appendix 3.4.

### Sludge after Disinfection and Sludge from Digester

Sludge was sampled directly from taps of the transfer pipes (Picture 3.5). Aliquots were collected in metallic containers. The composite sample was cautiously homogenized before transferring the required quantity for the analysis into glass jars.

### Supernatants from the Sludge Storage Tank

Supernatants from the sludge storage tank are returned to the primary settling tank about five times a day. Aliquot samples were taken approximately every ten minutes during the first transfer in the morning. They were collected in a glass bottle, which was protected from sunlight during sampling. Since sludge in the storage had remained previously for about one month in the digester that is mixed continuously, it can be expected that concentrations in sludge and supernatants from the storage tank do not vary substantially. It is therefore justifiable that samples were taken in the morning only.



### Sludge from Stock

Samples were taken from different depths of both sludge storage tanks after the supernatants had been pumped to the primary settling tank (Picture 3.6). Samples were collected from the storage tank with a sampling bottle at 6 points from the bridge of each of the two storage tanks. The 12 aliquots were collected in a metallic container. The composite sample was cautiously homogenized before transferring the required quantity for the analysis into glass jars. Sludge in the storage tank was not mixed before sampling.



Picture 3.6

Every liquid sample taken had at least a volume of eight litres in order to ensure a representative sampling and because samples were collected for other research purposes as well. The volume of sludge samples was between 20 and 30 litres approximately. All samples were protected from sunlight and cooled immediately after sampling and during transport. Devices and containers used for sampling supernatants and sludge were made of aluminium and were carefully rinsed with tap water before use. During all operations contact of samples with plastic was strictly avoided to prevent contamination. After use all containers for sample collection were washed with washing up liquid (Migros, Handy, Art. no. 5010.005) and rinsed with acetone and hexane afterwards in order to avoid contamination.

After mixing homogeneously all samples were stored in aluminium container of 0.4 or 0.9 litres (Article-Nr. 7045.404/5, Migros) immediately after arrival in the Laboratory. These containers were covered with a cardboard lid and protected by a plastic bag. Samples were stored in the freezer at  $-20^{\circ}\text{C}$ . No preservatives or stabilisers were added and if samples were joined in order to reduce the number of analysis, this was accomplished after defrosting just before extraction.

### 3.3 Analytical Method

The analytical method was adapted from the method described by Herren et al. (2000) to the analysis possibilities in the Laboratory for Environmental Chemistry and Ecotoxicology (CECOTOX) at the Swiss Federal Institute of Technology in Lausanne.

#### 3.3.1 Sample Characterisation

Samples were defrosted for 15 hours approximately at 20 °C. Normally sample's temperatures were still cooler than 20°C when extractions were started. It was assessed that freezing does not change polycyclic musk concentration by analysing a waste water sample that was frozen for 50 hours and a no frozen sample. Both samples were taken from the discrete waste water sample, sampled the last day of the sampling period (Appendix 3.5). The effect of freezing could not be assessed fully for sludge, which is the reason why sludge analysis were put back.

#### 3.3.2 Compound Abbreviations and Trade Names

Polycyclic musks identified are the following: HHCB (1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyran (Galaxolide, Abbalide, Pearlide), AHTN: 6-Acetyl-1,1,2,4,4,7-hexamethyl tetralin (Tonalide, Fixolide), ADBI: 4-Acetyl-6-tertbutyl-1,1-dimethylindane (Celestolide, Crysolide), AHMI: 6-Acetyl-1,1,2,3,3,5-hexamethylindane (Phantolide), AITI: 5-Acetyl-3-isopropyl,1,1,2,6-tetramethylindane (Traseolide), DPML: 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one (Cashmeran) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benuopyran-1-one (Galaxolidone). Galaxolidone, has been named and identified in the environment for the first time by (Franke et al., 1999) as an product of autooxidation of Galaxolide (Berset, 2002).

#### 3.3.3 Standards, Chemicals and Solvents

All polycyclic musks standards were purchased from Prochem GmbH, Wesel, Germany. Their purity varied between 74 % for Galaxolide and 98% for Celestolide and Tonalide. As Ford (1998) explains Galaxolide is commonly sold and used as an approximately 65% solution in neutral solvent because of its high viscosity. Galaxolidone was made available by J.-D. Berset (Gewässer- und Bonenschutzlabor, Kanton Bern, Switzerland). It was originally synthesised as described in Berset et al. (2002) by J.-D. Berset in collaboration with the group of Prof. P. Bigler from the Institute for Chemistry and Biochemistry of the University in Berne (Switzerland). Its purity was estimated 90% by Dr. Luc Patiny, EPFL Lausanne (Switzerland) by NMR- Analysis. Concentrations found in environmental samples were corrected by the purity of the standards.

An internal standard (Tonalide D3) to account for variation of the GC-MS was added to every sample before analysis. This internal standard was obtained from the Labor Dr. Ehrendorfer-Schäfers, Augsburg, Germany. N-Hexane (95%), isooctane and dichloromethane (DCM) were supplied by Romil, Amman-Technik AG, Köliken, Switzerland. Sodium Chloride (NaCl) and Sodium Sulphate (Na<sub>2</sub>SO<sub>4</sub>) were obtained from Merck KGaA, Darmstadt, Germany. Hexane (95%) and Acetone (99%) to rinse glassware was originally purchased from Fluka Chemika, Buchs, Switzerland and distilled twice in the laboratory before reuse.

#### 3.3.4 Sample Extraction

Water (treated waste water 600ml, water after primary settling 600 ml, treated waste water 700 ml, supernatants 600 ml, n=1) was extracted three times with 50 ml hexane in a seperatory funnel by shaking vigorously. Although phase separation was improved by adding 20g of NaCl, the two phases did not separate completely and the emulsion part had to be centrifuged (10 min at 3000 turns). The organic phase was decanted and dried over Na<sub>2</sub>SO<sub>4</sub>. 0.5 ml isooctane was added and the extract was concentrated at 40°C and 330 mbar to a volume of about 0.5 ml. The extract was diluted with a mix of dichloromethane: hexane (50:50 v/v) up to 5 ml and the solution further used for gel permeation chromatography (GPC) clean-up. The entire analysis method is outlined in Appendix 3.6.

### 3.3.5 Clean-up: Gel Permeation Chromatography (GPC)

It was found that a clean up process was important in order to obtain stable results in the GC-MS analysis. Gel permeation chromatography was chosen as a clean-up process. Solid Phase Extraction (SPE) (0.5g SiOH Chromabond columns from Macherey-Nagel, Düren, Germany, conditioned with 10 ml DCM and after loading eluted with 10 ml DCM) was tested as clean-up method for water samples, but it was found that the samples still contained substantial contamination when injected to the GC-MS. Furthermore GC-MS results obtained from samples cleaned up by SPE were found not to be stable, meaning that if a sample was injected several times different concentrations were found (Appendix 3.7).

Gel permeation chromatography system consisted of a Perkin-Elmer, Serie 4 pump with a 5ml sample loop, a column (600mm x 20 mm) that was filled with 100 g of Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) and a Gilson fraction collector, model 202. N-Hexane: Dichloromethane (50:50 v/v) was used as eluent at a flow rate of 5 ml/min. The optimum sampling window was between 45 and 80 min (225 and 400 ml) (Appendix 3.12). 0.5 ml isooctane was added to the GPC eluates and it was evaporated at 40°C and 750 mbar. Subsequently the vacuum was increased to 550 mbar and 330 mbar in order to evaporate hexane up to about 0.5 ml. The extract was transferred to a vial and rinsing of the original flask was added to the vial. The extract was concentrated under a gentle stream of nitrogen up to about 0.5 ml. Careful evaporation was crucial and could easily be monitored by the recovery rates of Cashmeran (DPMI) as shown in Appendix 3.8. Extracts were diluted with isooctane up to 1 ml.

### 3.3.6 Quantification GC-MS

0.1 ml of the internal standard (Tonalide D3) at a concentration of 4 µg/g was added to 0.4 ml of the final extract in order to assess the variation of the GC-MS. Quantification was conducted with a HP 5890 - 5971A MSD GC-MS with a DB-5ms (50m x 0,20mm x 0,33µm) column, series 9987123. Analysis was run in SIM modus. The characteristic ions as retention and starting times are reported in Appendix 3.9. Since the peaks of Galaxolide, Tonalide and Tonalide D3 were not clearly separated, a specific ion was extracted for each of these compounds to analyse them. These masses were 213 (26 %) for Galaxolide, 258 (31 %) for Tonalide and 261 (31 %) for Tonalide (D3). It was found that the GC-MS had to be stabilised with an environmental sample, if not, peak areas of standard solutions varied considerably as can be seen in Appendix 3.10.

Temperature of the oven was increased as following: 80°C for 1 min, 10°C/min up to 150°C, 2°C/min up to 280°C. If standard solutions were analysed, temperature was kept at 280°C for 17 min, which resulted in 90 min analysing time. For environmental samples temperature was held at 280°C for 5 min only, and then it was raised by 2°C/min to 300°C. This temperature was kept for 25 min in order to clean the column. (for temperature protocols see Appendix 3.9) The two temperature programmes did not change retention times, since all compounds were eluted before the programmes differ. The temperature of the splitless injector was kept constant at 300°C. It was found that a higher injector temperature increased the detection limit of Galaxolidone considerably. (Appendix 3.11) but due to material restriction temperature could not be risen higher than 300°C. Polycyclic musk concentrations were determined by comparing the peak areas of environmental samples with the peak areas of a mixture of standard solutions with the following concentrations: Cashmeran: 0.03 µg/g, Celestolide: 0.14 µg/g, Phantolide: 0.07 µg/g, Traseolide: 0.12 µg/g, Galaxolide: 13.74 µg/g, Tonalide 2.33 µg/g and Galaxolidone: 0.23 µg/g. Concentrations of the standard mixture were determined by analysing the discrete waste water sample taken at the last day of the sampling period. This sample was not frozen, but refrigerated. It was found that the concentrations in this sample above all the one for Galaxolide were considerable higher than the concentrations found in continuous samples. There may have been a wave of detergent entering the waste water treatment plant at the moment the sample was taken.

### 3.3.7 Repeatability, Recovery Experiments and Detection Limits

A water sample (discrete sample, taken at the last day of the sampling period) was extracted and analysed several times during the establishment of the analysis method. Concentrations found are stable as illustrated in Table 3.2

Table 3.2: Polycyclic musk concentrations (ng/l) found in a discrete waste water sample, extracted and analysed several times

	extraction 20.06.02 injection 26.06.2002 (SPE/GPC)	extraction and injection 27.06.02 (GPC)	extraction and injection 2.7.02 (GPC)	mean	standard deviation (% of the mean)
no. Analysis	26060204	26060218/20	2070205		
Cashmeran	31.13	21.90	31.39	28.14	19%
Celestolide	145.03	170.86	166.53	160.81	9%
Phantolide	87.69	72.42	75.16	78.42	10%
Traseolide	132.25	142.10	135.36	136.57	4%
Galaxolide 213	13119.55	13778.12	14403.64	13767.10	5%
Tonalide 258	2763.48	3041.85	2842.05	2882.46	5%
Galaxolidone	230.58	104.19	223.30	186.02	38%

Two different values were found for Cashmeran and Galaxolidone in the second sample. Unfortunately it could not be assessed why these two values were lower. There might have been a problem in the GPC clean-up process, since these two compounds are eluted at last (Appendix 3.12). If these values are not taken into account to calculate the mean and the standard deviation, the relative standard deviations are 1% for Cashmeran and 2% for Galaxolidone only.

Due to time restriction one recovery experiment was carried out only. A discrete waste water sample, taken the last day of the sampling period, was spiked with 50, 100, 150, and 200% of the amount of polycyclic musks it contained. Recovery rates, their standard deviations and random deviations of the straight line can be obtained from Table 3.3

Table 3.3: Recovery rates and their standard deviation as the random mistake assessed by spiking a water sample

	recovery rate	standard deviation of recovery rate	random deviation of the straight line (ng/l)
Cashmeran	97.3%	1.61%	1.39
Celestolide	99.9%	4.89%	15.81
Phantolide	92.9%	4.12%	4.74
Traseolide	91.2%	4.69%	13.33
Galaxolide 213	86.3%	3.34%	910.33
Tonalide 258	86.7%	4.26%	256.63
Galaxolidone	92.9%	2.07%	39.16

Graphs of the spiking experiment for each compound are reported in Appendix 3.13. Recovery rates as their standard deviations are satisfactory and concentrations measured in the extract were corrected by recovery rates.

Detection limits were assessed by diluting the standard solution until the heights of the peaks found in the GC-MS were about three times the height of the noise. These experiments were carried out before analysing environmental samples. Concentrations computed and the corresponding detection limits are reported in Table 3.4.

Table 3.4: Detection limits derived from the analysis of standard solution

	DPMI Cashmeran	ADBI Celestolide	AHMI Phantolide	AITI Traseolide	HHCB Galaxolide	AHTN Tonalide	Galaxolidone
limit of detection pg/ $\mu$ l extract	2.20	1.95	1.24	2.42	10.51	7.77	15.09
limit of detection ng/l water	4.58	4.07	2.58	5.03	21.90	16.19	31.44

However as described above, it was found that the sensitivity of the GC-MS changes considerably if environmental samples were injected, meaning that the peak areas of standard solutions injected before and after an environmental sample varied significantly. This variation could be lowered to maximum 5 % if the GC-MS was stabilised with an environmental sample before analysis. Detection limits were assessed again. They were extrapolated from standard solutions injected to quantify environmental samples, since due to time limitation and restriction by the GC-MS no further injections could be accomplished. Results are summarized in Table 3.5

Table 3.5: Detection limits extrapolated from standard solutions injected in the GC-MS after stabilisation with an environmental sample

	DPMI Cashmeran	ADBI Celestolide	AHMI Phantolide	AITI Traseolide	HHCB Galaxolide	AHTN Tonalide	Galaxolidone
limit of detection pg/ $\mu$ l extract	5.94	5.30	4.69	6.12	8.05	5.72	4.88
limit of detection ng/l water	12.38	11.04	9.76	12.75	5.72	11.91	10.16

As can be seen, different detection limits were found with or without stabilisation. In order to ensure, whether the concentrations found in the samples exceeded detection limits always, it was controlled that all peaks found were three times higher than the noise to their sides. This was the case for every peak except once for Galaxolidone and therefore it can be concluded that the concentrations found in the samples lay above the detection limits.

### 3.3.8 Contamination of the Laboratory by Polycyclic Musks

Due to the widespread use of musks, it is important to monitor contamination of the laboratory, in order to track possible contamination. Therefore hexane used for the last rinsing of the glassware was analysed as the environmental samples. In none of these blank samples contamination by polycyclic musks could be found. Even though no contamination was found up to now, it still has to be monitored in future analysis.

## 3.4 Evaluation Methods

### 3.4.1 Methodology to Represent Musk Pollution Load

Waste water entering a waste water treatment plant origins from different sources such as private households, industry and run-offs. This can result in different types of waste water, which were classified by (Kupper et al., 1999). If waste water originating from households is diluted considerably by run-offs, a much lower concentration of polycyclic musks can be expected than in undiluted water since fragrances are discharged primarily from households. Industrial waste water could have a dilution effect as well, since, caused by the specific use of polycyclic musks in cosmetic products and detergents, it is expected that these compounds are mainly found in domestic waste water (Berset et al., 2002). Due to these dilution effects, it is difficult to compare concentrations of polycyclic musks measured in waste water in different treatment plants. However there are hardly ever indications of the weather conditions during sampling period or description of the catchment's areas (number of households connected to the plant, type of industry connected) when concentrations of polycyclic musks are published and therefore one is often obliged to compare concentration nevertheless.

However more information is available on measurements of polycyclic musk concentrations carried out within the SEA project in the waste water treatment plant in Chevilly (CH) and Konolfingen (CH) about a year ago. There are 210 inhabitants connected to the plant in Chevilly, whereas the waste water treatment plant 'Mittleres Emmental' services 22'716 and Konolfingen 7861 respectively. Furthermore inflow rates were measured in all three plants, which assesses for dilution by run-offs. This information allowed calculating the flows (mg/y/cap) of polycyclic musks entering and leaving the plant. Furthermore sludge production rates and dry matter content of sludge were measured, which enabled to assess the polycyclic musk load in sludge as well. Since it is considered as more adequate to compare flows instead of

concentrations, flows in different SEA waste water treatment plants are contrasted in the discussion.

### 3.4.2 Transfer Coefficient

Transfer coefficients estimate the percentage removal of a compound during a process and they are calculated by dividing the output concentration or flow by the input concentration (or flow) and subtracting the result from one, as shown in the following formula:

$$\text{Transfer Coefficient} = 1 - \frac{\text{conc}_{\text{output}}}{\text{conc}_{\text{input}}} = 1 - \frac{\text{flow}_{\text{output}}}{\text{flow}_{\text{input}}}$$

Transfer coefficients are used in material flux analysis as described by (Baccini et al., 1996) and are useful to assess percentage removal. Therefore transfer coefficients are computed for all compounds in primary settling, secondary treatment and for the entire waste water treatment process.

### 3.4.3 Statistical Methods

There are always uncertainties in an analysis method and hence the concentrations measured have to be seen as an approximation of the exact value. The variation was assessed by the results of the spiking experiment, since there were not enough replicates of one single sample to determine variation and since the analysis method is too complicated to account for every uncertainty separately resulting in a calculation of errors. As advised by Reinhard Furrer (EPFL) the random deviation of the straight line assessed in the spiking experiment was taken as the standard deviation of the analysis method even though the condition of a correlation of zero was not fulfilled. Correlation is not zero since the concentrations found in the spiked samples are not independent due to the original waste water that already contained a certain amount of musks. However it gives an idea of the variation of the analytical method. Random deviations of the straight line can be found in Table 3.3

### 3.4.4 Calculation of Polycyclic Musk Concentrations in Sludge from Mass Balance

Polycyclic musk concentrations in sludge could not be analysed due to time restriction and analytical problems. Therefore concentrations in activated and primary sludge were estimated by balancing fluxes within the waste water treatment plant, assuming no degradation within the plant and no losses in the sand trap and by screening. Van de Plassche et al. (1997) assumed also a biodegradation rate of  $0 \text{ hr}^{-1}$ , this as a conservative approach due to a poor data basis. However this is not in accordance with findings published by Balk et al. (1999), who assessed primary biodegradation rate constants in activated sludge for Galaxolide and Tonalide of  $0.067 \text{ h}^{-1}$  and Artola-Garicano (2002), who found biodegradation rate constants of  $0.071 \text{ h}^{-1}$  for Galaxolide and  $0.023 \text{ hr}^{-1}$  for Tonalide. In order to assess which of the statements suits better to the data found in the waste water treatment plant 'Mittleres Emmental', polycyclic musk concentrations in activated, in primary and in digested sludge have to be analysed. This report is focused on the calculation of the expected flows, measurements will be accomplished in a second phase. Concentrations of polycyclic musks in digested sludge are not computed since divergent publications on the reduction of polycyclic musks during digestion were found (Van de Plassche et al., 1997, Berset et al., 2002).

Polycyclic musk flows in activated sludge were calculated by balancing the masses entering and leaving the secondary settling tank as shown in the following formula. Musks flows were calculated for every day by multiplying concentrations with water flows. The expected flow in activated sludge was estimated by the mean of the daily flows. Results are reported in Chapter 5.1.

$$\text{Flow}_{\text{AS}} = \frac{\text{Flow}_{\text{WapS}} - \text{Flow}_{\text{TWW}}}{\left(\frac{\text{amount}_{\text{ES}}}{\text{amount}_{\text{AS}}}\right)}$$

AS: activated sludge  
 WapS: waste water after primary settling  
 TWW: treated waste water  
 ES: excess sludge

From the polycyclic musk flow/cap/year in activated sludge, expected concentrations can be calculated by multiplying the flow/cap/year with the number of inhabitants (22'716 for the waste water treatment plant 'Mittleres Emmental') and the amount of activated sludge produced a year. This value was extrapolated from the measurements carried out in the waste water treatment plant 'Mittleres Emmental' in April (amount of activated sludge pumped in April: 323000 m<sup>3</sup>, average dry matter content 0.71%) and the assumption of the density of the solids of 1.3 kg/l (Van de Plassche et al., 1997). Results can be found in Chapter 5.1.

Polycyclic musk flows in primary sludge were calculated by mass balancing the thickener, resulting in the following formula.

$$Flow\_PS = Flow\_SNS + Flow\_WW - Flow\_TWW$$

PS: primary sludge

SNS: supernatants from the sludge storage tank

WW: waste water

TWW: treated waste water

As for activated sludge polycyclic musk fluxes in primary sludge fluxes were calculated for every day of the week and the expected flow in primary sludge was estimated by the mean of the daily flows. Expected concentrations in primary sludge were computed by multiplying the flows/cap/year with the number of inhabitants (22716) and dividing it through the amount of primary sludge produced a year (973 t/dm, personal communication: B. Bangerter, GSA). As for activated sludge, results are reported in Chapter 5.1.

## 4. Results and Discussion

### 4.1 Concentrations in Waste Water, Water after Primary Settling and Treated Waste Water

Concentrations of polycyclic musks measured in waste water, water after primary settling and treated waste water in the waste water treatment plant 'Mittleres Emmental', Switzerland between the 9<sup>th</sup> and the 23<sup>rd</sup> of April 2002, Switzerland are reported in Figure 4.1.

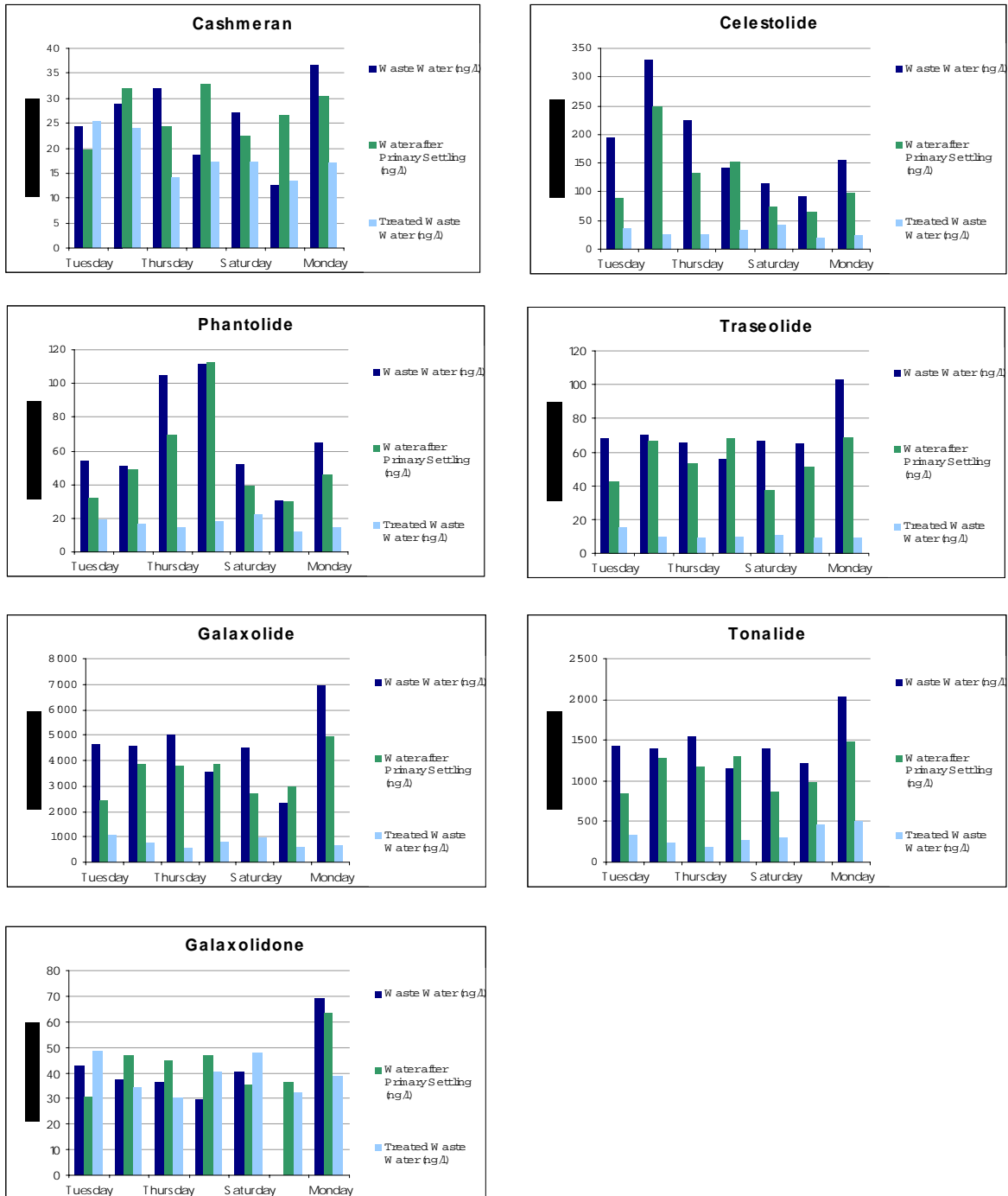


Figure 4.1

Concentrations of polycyclic musks in waste water, water after primary settling and treated waste water (ng/l)



Corresponding data can be found in Appendix 4.1 and mean values are reported in Table 4.1. Results were obtained by the analysis method described in Chapter 3.

Table 4.1: Mean polycyclic musk concentrations (ng/l) found in the waste water treatment plant 'Mittleres Emmental'

	Mittleres Emmental			
	waste water (ng/l)	water after primary settling (ng/l)	treated waste water (ng/l)	analytical uncertainty (ng/l)
Cashmeran	26	27	18	2.7
Celestolide	179	123	30	31.0
Phantolide	67	54	17	9.3
Traseolide	71	56	10	26.1
Galaxolide	4507	3505	780	1784.3
Tonalide	1454	1132	328	503.0
Galaxolidone	37	43	39	76.8

If random deviation, hence analytical uncertainty as explained in Section 3.4.3 and reported in Table 4.1 is taken into account, meaning that on a 95% confident level measured concentrations can vary  $\pm 1.96 \cdot$  random deviation = analytical uncertainty, no interpretation of the results is possible, since uncertainty is substantial after all in treated waste water where concentrations are low. However it should not be forgotten that measurements are never as precise as exact figures imply.

The concentrations of Galaxolide and Tonalide measured in waste water of the waste water treatment plant 'Mittleres Emmental' are between 4507 ng/L (2338–6937 ng/L) and 1454 ng/L (1150–2040 ng/L) respectively. The other compounds' concentrations including the metabolite Galaxolidone were in a much lower range (between 10 and 200 ng/L). The results of the waste water treatment plant 'Mittleres Emmental' can be compared with concentrations found in two other waste water treatment plants Chevilly and Konolfingen of the SEA network. Concentrations and percentage differences to the measurements in the waste water treatment plant 'Mittleres Emmental' are indicated in Table 4.2. The works in Chevilly and Konolfingen took place in April and Mai 2001. Waste water and treated waste water were analysed in Chevilly daily during a week whereas in Konolfingen a weekly composite sample of water after primary settling and treated waste water was examined. The treatment plant of Chevilly is of an extended aeration type, whereas in Konolfingen and 'Mittleres Emmental' sewage is treated with the activated sludge technique. Polycyclic musk concentrations in sewage are for most compounds higher in Chevilly in comparison with Konolfingen and 'Mittleres Emmental' (for Galaxolide: 6901, 1682 and 4507 ng/L respectively). These differences can be explained by the drainage system and the catchment's area. Chevilly has a separated drainage system and no industry in the catchment's area. Hence domestic sewage is not diluted by stormwater or industrial wastewater. The differences between 'Mittleres Emmental' and Konolfingen are due to rain events during sampling period in Konolfingen and high influent from food processing industry in this plant. For the dissimilar types of waste water and the differences in the catchment's area it is accounted by comparing musk fluxes per person and time as done in section 4.4.

Important differences in influent concentrations were observed for Galaxolidone, which concentration is by a factor 10 higher in Chevilly than in the waste water treatment plant 'Mittleres Emmental' even though Galaxolide concentrations are in the same range. It is difficult to assess this result. It may be an analytical problem, but it is not easy to find the cause, because recovery experiments for Galaxolidone were carried out with good recovery rates (93%) but if the GC-MS was not stabilised with environmental sample, the peak areas of Galaxolidone between different injections of the same sample varied considerably. Even though there is no increase in Galaxolidone concentration during the waste water treatment process in the waste water treatment plant 'Mittleres Emmental' as in the other plants, there is neither a decrease as observed for all the other compounds. This may indicate that there is still a degradation process of Galaxolide to Galaxolidone, hence a production of Galaxolidone. However it is not as significant as in the other two treatment plants. Celestolide concentrations of 179 ng/l are about twice as high in the waste water treatment plant 'Mittleres Emmental' than in Chevilly and Konolfingen. This high concentration results from the outstanding high influent concentration measured on Tuesday to Thursday. It can be assumed that on these days some special activities took place in the catchment's area, which led to an increased discharge of

Celestolide. Since no specific use of Celestolide could be found, it is not possible to relate the high concentrations to a potential event.

The analysis method was slightly different in this study (Chapter 3.3) than the method used in Chevilly and Konolfingen (Berset et al., 2002). However in summary it can be stated that concentrations found are comparable (Table 4.1, Table 4.2).

Table 4.2: Concentrations (ng/l) of polycyclic musks in water measured in two SEA waste water treatment plants and in two other studies. Percentage refers to difference between results obtained in WWTP Mittleres Emmental. WW: waste water, WapS: water after primary settling, TWW: treated waste water

	Chevilly				Konolfingen				6 WWTP in BL (1999) (mean)	17 WWTP (SAFEL, 1998) (median)	range of literature values	
	WW (ng/l)		TWW (ng/l)		WapS (ng/l)		TWW (ng/l)		TWW (ng/l)	TWW (ng/l)	WW (ng/l)	TWW (ng/l)
Cashmeran	33	28%	7	-62%	14	-48%	7.2	-61%	> 500		-	-
Celestolide	84	-53%	16	-47%	58	-53%	13.2	-56%	83	>100	40-290	40-430
Phantolide	195	191%	65	288%	64	18%	15.6	-7%	104		-	-
Traseolide	169	139%	19	82%	57	3%	14.3	37%	43		-	-
Galaxolide	6901	53%	857	10%	1682	-52%	255	-67%	2983	2300	500- 30'000	160- 11'000
Tonalide	1518	4%	247	-25%	600	-47%	94	-71%	1960	1400	800- 12'500	110- 5'800
Galaxolidone	426	1066%	899	2218%	276	538%	344	787%				

In other studies conducted in Switzerland higher polycyclic musk concentrations in treated waste water were found (last two columns in Table 4.2). It is difficult to account for the differences; dissimilar analytical methods might be an explanation, since according to the production rates no decrease in discharge can be assumed. Furthermore only median concentrations were found for the SAFEL study and comparison with mean concentrations is possible only if concentrations are distributed normally.

Comparing concentrations obtained in the three SEA waste water treatment plants with results found in the literature (Appendix 1.5), considerable differences can be observed. Galaxolide concentrations of about 10 µg/l for treated waste water are reported (Heberer et al., 1999), which is more than 100 times higher than the value found in this study (780 ng/l). The lowest concentration of Galaxolide in waste water treatment effluent was measured at 320 ng/l (Verbruggen et al., 1999), however only bioavailable fractions and not the total amount of musks were measured in this study. The concentrations for Tonalide measured in this study are in the lower range of concentrations published in the literature (Appendix 1.5), whereas if compared with results found within the SEA project Tonalide concentrations are in the same range. Only few values for Celestolide concentrations in waste water could be found in the literature. Celestolide concentrations measured in waste water in this study (179 ng/l) are in the upper range of values already detected, whereas treated waste water concentration (30 ng/l) is relatively low. Concentrations of Phantolide and Traseolide in waste water and treated waste water are reported in only two other publications than SEA reports. Compared to these values the concentrations found in this study are quite low. It is difficult to account for the different concentrations found. Analytical differences may be a reason, however since the original publications often cannot be consulted, it is difficult to compare the analytical methods. Furthermore catchment's areas and treatment techniques, which can be thought of influencing polycyclic musk concentrations, can differ considerably between different waste water treatment plants and if run-offs are not separated from waste water, weather conditions influence the concentrations substantially. Additionally it was found that concentrations of Galaxolide and Tonalide in surface water were 3-8 times higher in winter times than in summer (Wiegel et al., 2000), indicating that there is a seasonal variation as well. All these parameters induce a high uncertainty so that results from different studies must be compared carefully. Specific fluxes (per capita and time) are less sensitive to errors since dilution effects caused by stormwater or industrial wastewater are compensated. Additional information needed for such calculations is

available within the SEA project and polycyclic musk fluxes are computed and compared in Section 4.4.

## 4.2 Daily Variation within the Waste Water Treatment Plant

The highest concentrations of Cashmeran, Traseolide, Galaxolide, Tonalide and Galaxolidone in waste water are measured Monday. Since influent rate was not higher on that day, the higher concentrations can only be explained by an increased discharge of these musk compounds to waste water, hence an increased use of washing agents, detergents or cosmetic products on that day. Monday may be the typical washing day for the families living in the catchment's area. However this assumption would need to be verified by further measurements and a sociological survey, assessing peoples washing habits. The highest concentrations of Phantolide and Celestolide in waste water are measured Friday and Wednesday respectively. It could be assumed that these fragrances are used in different products than the ones with the peak concentrations on Monday for example in detergents used in small industries or cleaning agents used by professional cleaners. However it could also be interpreted as a random error.

The lowest influent concentrations for all compounds are measured on Sunday with the exception of Tonalide, for this compound it is the second lowest value. On that day the measured Galaxolidone concentration are even below the detection limit. Problems reported during sample progression (Appendix 3.4) were limited and do not account for the unusual low values. However incomplete mixing of the sample before storage might have been a reason. It is difficult to find an analytical cause for the low values, since this sample was extracted and analysed with the waste water samples taken on Saturday and Monday that do not show irregularities.

In water after primary settling the concentrations of Traseolide, Galaxolide, Tonalide and Galaxolidone remain highest on Monday, whereas the highest concentration of Cashmeran is measured on Friday. However Cashmeran concentration remains high on Monday. It is interesting that for Celestolide and Phantolide the peak days remain Friday and Wednesday respectively, indicating consistent chemical analysis and sampling method. The lowest concentrations in water after primary settling are measured on Tuesday for Cashmeran, Galaxolide, Tonalide and Galaxolidone. For Phantolide and Celestolide only the lowest concentrations remain on Sunday pointing again to an irregularity in Sunday's waste water sample.

Peak days in treated waste water change for all compounds except Tonalide. The highest values are measured on Tuesday for Cashmeran, Traseolide, Galaxolide and Galaxolidone. It can be assumed that the peak concentrations for these compounds origin from Monday, but normal retention time for water in the waste water treatment plant is about 6 hours, which would be too short to explain the displacement of the peak days. Furthermore it is difficult to compare the values measured on Monday with the ones measured on Tuesday since the two days did not follow each other, because one was the first and the other one the last day of the sampling period. Lowest concentrations found in treated waste water are not compared since sampling and analytical uncertainties are quite high if low concentrations are measured.

Relative standard variations (Appendix 4.1) are lowest for treated waste water if compared with waste water and water after primary settling, indicating that waste water treatment levels concentrations.

## 4.3 Development of Polycyclic Musk Concentrations during the Waste Water Treatment Process

As polycyclic musks are lipophilic, they are removed from the waste water with solids; hence concentrations decrease with the waste water treatment process. Transfer coefficients estimate the percentage removal during a process, in this case during the waste water treatment process.

Mean transfer coefficients for primary settling are between 17 and 30 % depending on the polycyclic musk compounds except Cashmeran and Galaxolidone (Appendix 4.3). An explanation for the low removal of polycyclic musks in primary settling may be that polycyclic musks are adsorbed to a higher percentage to small sized suspended matter, that does not

settle in the primary settling tank. As shown by a one-sided student's T-test the decrease of polycyclic musk concentrations during primary settling is even not significant on a 95% level except for Traseolide and Tonalide (Appendix 4.2). The significant removal of the latter compounds may be explained by the relatively high  $K_{ow}$  values (Appendix 1.4), meaning that a higher percentage sticks to organic matter that settles down and is removed. However differences in  $K_{ow}$  values between the different compounds are not significant enough to account for the dissimilar transfer coefficients and furthermore  $K_{ow}$  value for Traseolide has never been measured but calculated only. The mean transfer coefficients for Cashmeran and Galaxolidone are negative, meaning that the concentrations of these compounds are rising during primary settling. This increase is significant on a 95% level for Galaxolidone, whereas for Cashmeran the increase is not significant on that level. Since Galaxolidone is a metabolite of Galaxolide the explication is easy to find, whereas it is more difficult to find an explanation for Cashmeran. The low concentration results in a high uncertainty with regard to analysis and homogeneity of the samples. Other causes may be the relatively low  $K_{ow}$  value (Appendix 1.4) of Cashmeran, meaning that compared to the other polycyclic musk compounds a lower percentage sticks to organic matter and is removed by sludge settling from waste water. The unusual low Cashmeran concentration of Sunday's waste water sample that results in a transfer coefficient of -109% for that day has also a considerable impact on the mean transfer coefficient. The transfer coefficients for Galaxolide and Tonalide found in the waste water treatment plant 'Mittleres Emmental' are consistent with the results found by Simonich et al. (2002), who assessed a percentage reduction of Tonalide and Galaxolide in primary settling of 28.9%  $\pm$ 20.1 and 29.9%  $\pm$ 23.4 respectively.

Biological treatment including phosphor precipitation reduces polycyclic musk concentrations significantly for all compounds except the metabolite Galaxolidone (one sided t-Test on a 95% level) (Appendix 4.2). This is also represented by the transfer coefficients that are higher than in primary settling with values between 63 and 80%. Consequently it can be stated, that the whole waste water treatment process reduces polycyclic musk concentrations significantly except for the metabolite's concentration. Mean transfer coefficients for the entire waste water treatment process are reported in Table 4.3. The low transfer coefficient of Cashmeran (23%) in the waste water treatment plant 'Mittleres Emmental' results from the negative transfer coefficient in primary settling and the low transfer coefficient in biological treatment.

The relative increase of Galaxolidone is substantially lower in the waste water treatment plant 'Mittleres Emmental' than in Chevilly, which is due to the lower concentrations in the effluent. However the little differences between the transfer coefficients (10%) of the two plants are remarkable if the variations in concentrations measured in the different plants are taken into account. The transfer coefficients differ slightly more between the waste water treatment plant 'Mittleres Emmental' and Konolfingen, which can be accounted for by the fact that in Konolfingen a weekly composite sample was analysed, whereas in Chevilly daily samples were examined, which levels random fluctuation. Overall it can be stated that transfer coefficients found in this report are consistent with the results obtained in other Swiss waste water treatment plants monitored within the SEA project.

Table 4.3: Transfer coefficient waste water (WW) – treated waste water (TWW) for two waste water treatment plants 'Mittleres Emmental' and Chevilly and transfer coefficient water after primary settling (WapS) – treated waste water (TWW) for the waste water treatment plant 'Mittleres Emmental' and Konolfingen.

	Mittleres Emmental		Chevilly		Mittleres Emmental		Konolfingen
	mean TC (WW-TWW)	std deviation	mean TC (WW-TWW)	std deviation	mean TC (WapS-TWW)	std deviation	TC (WapS-TWW)
Cashmeran	23%	26%	77%	8%	29%	28%	49%
Celestolide	80%	10%	80%	6%	70%	15%	77%
Phantolide	71%	11%	61%	15%	63%	17%	76%
Traseolide	85%	4%	87%	5%	80%	8%	75%
Galaxolide	81%	6%	87%	4%	76%	11%	85%
Tonalide	77%	8%	83%	7%	70%	12%	84%
Galaxolidone	1%	28%	-111%	46%	4%	37%	-25%

Entire waste water treatment process transfer coefficients for Galaxolide (81%) and Tonalide (77%) are also in accordance with results published by Simonich et al. (2002), who found percentage removal in primary gravitational settling and activated sludge treatment of  $88.8 \pm 6.3$  % for Tonalide and  $87.8 \pm 7.9$  % for Galaxolide. However other results were published also, Eschke et al. (1994) analysed Galaxolide and Tonalide in influents and effluents of sewage treatment plants and found relatively high concentrations in effluent samples indicating only poor retention.

Simonich et al. (2002) conclude from their analysis of different fragrance materials including Tonalide and Galaxolide that the removal of sorptive, nonbiodegradable fragrance materials in a waste water treatment plant correlate with the removal of total suspended solids. It would be interesting to determine the removal of total suspended matter in primary and secondary settlement in the waste water treatment plant 'Mittleres Emmental' in order to assess if a correlation can be found for the other polycyclic musk compounds as well. A correlation is expected since  $K_{ow}$  values are similar for all compounds.

#### 4.4 Comparison between Measured and Calculated Flows

Within the SEA Project the analytical methods did not differ considerably (Herren et al., 2000) and in order to account for metrological differences during the sampling period and possible dilution by industrial waste water, concentrations are multiplied by the water flows (Table 3.1) resulting in the musk flux per time. The differences in the catchment's area are taken into account by dividing the fluxes by the number of inhabitants connected to the waste water treatment plant. In Chevilly these are 210, in Konolfingen 7861 and in the area of the waste water treatment plant 'Mittleres Emmental' 22'716. The polycyclic musk flows per capita and day are represented in Table 4.4

Table 4.4: Polycyclic musk flows per capita and day ( $\mu\text{g}/\text{cap}/\text{day}$ ) in three SEA waste water treatment plants and percentage difference to the waste water treatment plant 'Mittleres Emmental', WapS: water after primary settling

	Mittleres Emmental			Chevilly				Konolfingen			
	waste water	water apS	treated waste water	waste water		treated waste water		water apS		treated waste water	
Cashmeran	9	10	6	5	-41%	1	-82%	3	-63%	2	-72%
Celestolide	62	44	11	14	-77%	3	-75%	14	-67%	3	-70%
Phantolide	24	19	6	34	43%	11	82%	16	-17%	4	-35%
Traseolide	25	20	4	28	12%	3	-14%	14	-28%	4	-3%
Galaxolide	1'591	1'239	277	1'170	-26%	143	-48%	418	-66%	63	-77%
Tonalide	510	400	114	251	-51%	41	-64%	149	-63%	23	-80%
Galaxolidone	13	15	14	71	446%	149	983%	69	347%	85	522%

In general the flows between the three waste water treatment plants are comparable. The value for Galaxolidone in the waste water treatment plant 'Mittleres Emmental' is much lower due to the considerable lower concentrations measured in this plant. Tonalide and Galaxolide flows per person are quite low in Konolfingen ( $418 \mu\text{g}/\text{cap}/\text{day}$  and  $150 \mu\text{g}/\text{cap}/\text{day}$ ) compared to the waste water treatment plant 'Mittleres Emmental' ( $1600 \mu\text{g}/\text{cap}/\text{day}$  and  $510 \mu\text{g}/\text{cap}/\text{day}$ ). However results of Konolfingen must be interpreted carefully as mentioned above. Percentage variations between the waste water treatment plants are different if flows and not concentrations are considered (Table 4.2 and Table 4.4). However there is a stable factor relating the variations of two plants since the factor relating concentrations and flows is stable.

Transfer coefficients do not change if flows and not concentrations are assessed because the inflow and the outflow rate usually do not differ considerably in a specific waste water treatment plants.

Flows computed from measured concentrations can be compared with flows calculated from consumption. It is interesting to compare the flows per capita with the use of polycyclic musks in Europe. Actually Galaxolidone flows would have to be added to Galaxolide flows in order to compare it with the production data but since Galaxolidone flows are very little, this does not change the result substantially.

Flows calculated from production rates are lower as the one computed from the concentrations measured except for Celestolide, where almost 100% is found in waste water and Traseolide for which much more is detected than produced Table 4.5. The production of Traseolide decreased considerably from 1995 to 1998 (Table 1.2) and it can therefore be assumed that there is still a stock on Traseolide that is released now. About a third only of the expected Tonalide, Galaxolide and Phantolide flow can be detected in the waste water treatment plant investigated. This is in accordance with other studies (Berset et al., 2002) and the risk assessment conducted by Balk et al. 1999b. The differences can be explained by a degradation process along the way to the waste water treatment plant, production rates that are estimated too high due to the fact that weight of carriers is not subtracted from the total amount sold (as explained in Section 3.3 carrier substances can make up to 30% of the weight) or other ways musks are discharged to the environment for examples by air. Evidence for this contamination way has been found by Kallenborn et al. (1999), who detected polycyclic musks, even at low concentrations, in Norwegian air samples. The pollution path in this case would be by evaporation from skin and textiles. However this is controversial due to the low vapour pressure of polycyclic musk compounds.

Table 4.5: Use of polycyclic musks in Europe and the proportion found in waste water

	use per year (t) in Europe	use per year per capita ( $\mu\text{g}$ ) <sup>3</sup>	proportion found in waste water
Celestolide	18 <sup>1</sup>	25	96%
Phantolide	19 <sup>1</sup>	26	34%
Traseolide	2 <sup>1</sup>	2.8	344%
Galaxolide	1427 <sup>2</sup>	1965	35% <sup>4</sup>
Tonalide	343 <sup>2</sup>	472	41%

<sup>1</sup> Source: value of 1998, (OSPAR Commission, 2000)

<sup>2</sup> Source: value of 2000, A. Weber (SAFEL), Personal communication, source IFRA

<sup>3</sup> calculate from the total use in Europe (number of Europeans 725'962'762, Microsoft Encarta, Weltatlas 2000)

<sup>4</sup> Galaxolide and Galaxolidone

Predicted environmental concentrations (PEC) are estimated assuming that 100% of polycyclic musks used are discharged to waste water, including a safety factor of three and supposing a sewage discharge of 200 l/d/cap (Van de Plassche et al., 1997). This results in a PEC value of Galaxolide in waste water of 160  $\mu\text{g/l}$ , which is about 40 times higher than the measured concentration in the waste water treatment plant 'Mittleres Emmental'. Outflow PEC for Galaxolide differs about the same factor from the actual measured value, resulting in a transfer coefficient that is in accordance with the results in this study. The differences between the PEC and actually measured values are not much smaller if the Galaxolidone flow is added to the Galaxolide flow. The predicted waste water concentration (PEC) of Tonalide computed, as for Galaxolide is about a factor 45 higher than the measured concentration, the same is true for treated waste water concentration. This follows in a transfer coefficient for Tonalide that is similar to the one found in the waste water treatment plant 'Mittleres Emmental'. Predicted environmental concentrations for the other polycyclic musks compounds were not found in the literature but they were computed using the same estimation methods as van de Plassche et al. (1997) Table 4.6.

Even if PECs for waste water treatment plants influents are predicted without a safety factor, with the actual number of inhabitants in Europe (Microsoft Encarta Weltatlas 2002), which is about twice as high as the number used by Balk, more recent production rates (1998) and average sewage discharge per capita as measured over five year within the SEA waste water treatment plants (440 l/day/cap), one obtains PEC values much higher than the one measured within this study Table 4.6. Reasons for these differences are the same as outlined for variation between expected and measured flows.

Table 4.6: PEC values for waste water (ng/l) computed according to van de Plassche et al. (1997), and according to this study (without a safety factor, with more recent production rates (1998) and higher number of inhabitants and inflow rates per capita measured within the SEA project)

	van Plassche *	this study °	inflow 'Mittleres Emmental'
Celestolide	3'776	155	179
Phantolide	5'553	163	67
Traseolide	4'443	17	71
Galaxolide	164'606	12'247	4'507
Tonalide	64'976	2'948	1'454

\* production rates of 1995, Table 1.2, inhabitants of Europe:  $3.7 \cdot 10^8$ , sewage production per day and capita: 200l, safety factor 3

° production rates of 1998 or where available 2000 Table 1.2, inhabitants of Europe  $7.25 \cdot 10^8$ , sewage production per day and capita 440 litres, without safety factor

#### 4.5 Correlation of Concentrations of Polycyclic Musk in Waste Water, Water after Primary Settling and Treated Waste Water

A stable relation between different polycyclic musk compounds could be expected, since companies buy mixtures of musks fragrances to perfume their products and it can be thought that these mixtures contain the same proportion of the different musk compounds. This would result in a stable proportion in waste water. Coefficients of determination for a linear relationship were calculated for all polycyclic musks compounds in waste water, water after primary settling and treated waste water and are outlined in Appendix 4.4. Coefficients are too low except for Galaxolide and Galaxolidone to assume a correlation between different polycyclic musk compounds. It is not surprising that there may be a correlation between Galaxolide and Galaxolidone since Galaxolidone is a metabolite of Galaxolide. However this result is not confirmed by measurements accomplished in Chevilly (Berset et al., 2002). The fact that there is only a weak correlation between the compounds is confirmed by data found in the literature. Proportions of polycyclic musks found in the environment are outlined in Appendix 1.4.

It was estimated also, if there is a correlation between the concentration of a polycyclic musk compound in waste water and its concentration in water after primary settling or treated waste water. Correlation coefficients found are low (Appendix 4.4), meaning that it is difficult to consider transfer coefficients as stable. Transfer coefficients might depend on waste water concentration, which is assessed in the following paragraph.

#### 4.6 Correlation between Polycyclic Musk Concentrations in Waste Water and the Transfer Coefficients Waste Water- Treated Waste Water

It has been assumed, that there might be a correlation between the polycyclic musk concentration in waste water and the transfer coefficient from waste water to treated waste water (Berset et al., 2002). It is interesting that for some compounds the correlation coefficient ( $R^2$ ) calculated from the results obtained in this study (Table 4.7) is quite high, which would account for such a relation.

Table 4.7: Correlation coefficient and slope of the relation between waste water concentration and transfer coefficient between waste water and treated waste water

	R2	slope
Cashmeran	0.71	2.7
Celestolide	0.61	0.09
Phantolide	0.8	0.34
Traseolide	0.51	0.19
Galaxolide	0.75	0.004
Tonalide	0.04	0.005
Galaxolidone	0.23	0.6

However for Tonalide and Galaxolidone with a correlation factor of 0.23 and 0.04 such a relation has to be neglected. As there is a degradation of Galaxolide to Galaxolidone it is evident that correlations for these compounds are low but Galaxolide shows the highest correlation coefficient. It can be concluded, that there might be a correlation between waste water concentration and transfer coefficient, meaning that the transfer coefficient is dependent of the

inflow concentration, which would account for a concentration dependent removal process. It could be thought of an at least first order degradation process, which results in a quicker degradation if initial concentrations are higher or in an unstable removal of suspended matter, which would result in unstable transfer coefficients as well. Removal of suspended matter would have to be measured in order to account for this hypothesis.

#### 4.7 Supernatants

Polycyclic musk concentrations measured in supernatants of the thickener and the sludge storage tank are reported in Figure 4.2. Exact values can be found in Appendix 4.1. Polycyclic musk concentrations in supernatants always exceed concentrations in waste water. This is not surprising since musk concentrations in sludge, the origin of supernatants, are expected to be considerably higher than in waste water. The highest concentrations in supernatants from the thickener for all polycyclic musk compounds are measured in the joint sample of Tuesday to Thursday. As outlined in the sample progression report (Appendix 3.4), there was some sludge added to the supernatants on Tuesday, which would explain the higher concentrations. Concentrations in supernatants from the thickener vary substantially during the week (Figure 4.2, which might be rather due to irregularities in sampling as seen in the joined sample of Tuesday to Thursday because correlation between waste water concentrations and concentrations in supernatants is difficult to establish. Due to these fluctuations, it is suggested that in a following study, samples of supernatants from the thickener should not be joined and should be analysed separately. However if it is aimed at a material flux analysis a mean value is considered as sufficient due to the low flux based on the little supernatants produced.

Supernatants from the two sludge storage tanks are pumped to the primary settling tank about four times on a weekday. Removal of supernatants reduces the volume of sludge for disposal. In general the concentrations in supernatants from the sludge storage tank are lower than the concentrations found in supernatants from the thickener Figure 4.2 and Table 4.8. The exception is Cashmeran. The general lower concentrations could be explained by a degradation process of polycyclic musk compounds during digestion as reported by van Plassche et al. (1997), who found a reduction in sludge of Galaxolide during digestion of 45% and for Tonalide of 40%. Another cause could be an improved separation of the solids from the liquids, which is accounted for by the higher amount of supernatants produced per day in the storage tank than in the thickener. The hypothesis of degradation during digestion will be verified in the second phase of this project, when sludge will be analysed. Furthermore concentrations in the supernatants from the sludge storage tank vary less than concentrations in supernatants from the thickener. This is evident taking into account that sludge in the storage tanks remained in the digester for about 30 days, where it was mixed well with sludge originating from other days. If resources are tight, it can be considered sufficient to analyse supernatants from the storage tank only once.

Table 4.8: Mean polycyclic musk concentrations (ng/l) measured in supernatants from the thickener and the sludge storage tanks in the waste water treatment plant 'Mittleres Emmental'

	supernatants from thickener (ng/l) (mean)	standard deviation	supernatants from storage tank (ng/l) (mean)	standard deviation
Cashmeran	44	25%	54	18%
Celestolide	339	43%	169	18%
Phantolide	152	31%	92	17%
Traseolide	169	34%	108	17%
Galaxolide	10529	26%	6374	17%
Tonalide	3503	30%	2187	17%
Galaxolidone	116	29%	107	11%



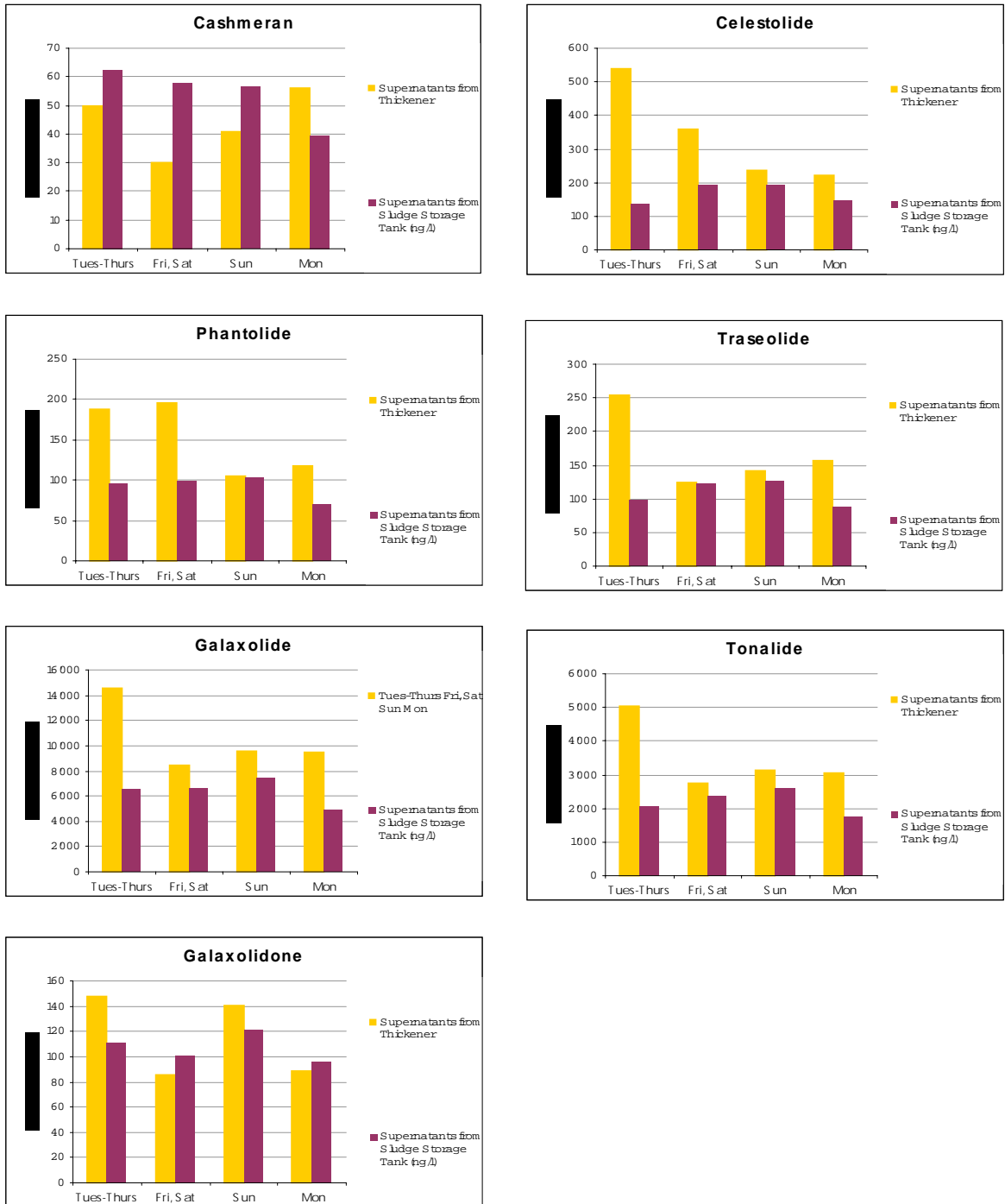


Figure 4.2

Concentrations of polycyclic musks measured in supernatants from the thickener and the sludge storage tank (ng/l)

### 5. Material Flux Analysis and Sludge Concentrations

The basis of a material flux analysis is flows. Polycyclic musks flows in the waste water treatment plant 'Mittleres Emmental' were calculated from concentrations and water flows measured and are reported in Figure 5.1. Exact values can be obtained from Appendix 5.1.

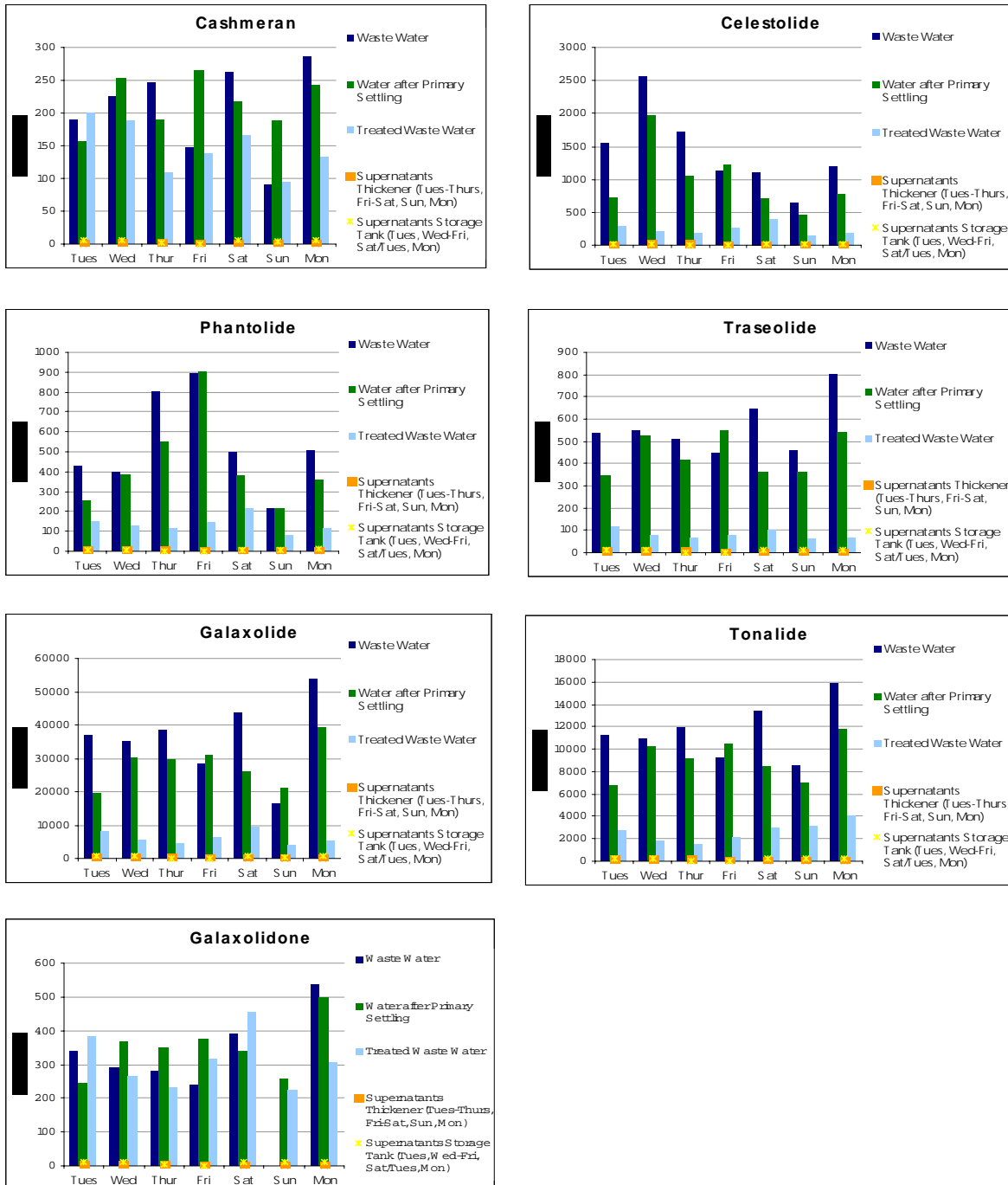


Figure 5.1

Polycyclic musk fluxes (mg/day) in the waste water treatment plant 'Mittleres Emmental'

Since influent, outflow of the primary settling tank and effluent rates do not vary substantially, the same pattern is found for flows as for concentrations: polycyclic musks fluxes decrease

within the waste water treatment process. Amounts transferred by supernatants are quite small. It can therefore be concluded that if resources are low and the aim of a project is the quantification of polycyclic musk fluxes within a waste water treatment plant, supernatants do not have to be analysed because their impact on the over all fluxes is negligibly small.

### 5.1 Estimation of Polycyclic Musk Concentrations in Sludge

Polycyclic musk flows in sludge can be calculated by balancing the masses in the waste water treatment plant as explained in Chapter 3.4.4. Galaxolide and Galaxolidone flows have to be added in order to track the total Galaxolide flow because degradation from Galaxolide to Galaxolidone is expected. Flows for the waste water treatment plant 'Mittleres Emmental' are reported in Table 5.1. Concentrations can be computed from fluxes as explained in Chapter 3.4.4. Results are summarized in Table 5.2.

Table 5.1: Expected sludge flows (mg/cap/year) in activated and primary sludge calculated from mass balances and actual primary sludge flows measured in Chevilly

	expected in activated sludge (mg/cap/year)	expected in primary sludge (mg/cap/year)	measured primary sludge Chevilly (mg/cap/year)	std deviation	expected primary sludge Chevilly (mg/cap/year)	measured/ expected
Cashmeran	98	1.0			1.5	
Celestolide	1057	18.9	3.1	0.6	4.3	-27%
Phantolide	430	6.5	13.3	2.6	8.4	59%
Traseolide	516	7.8	7.3	1.7	9.0	-19%
Galaxolide + Galaxolidone	31368	485.0	287.1	56.1	346.6	-17%
Tonalide	9414	146.4	96.3	21.3	76.5	26%

The most important flows of polycyclic musks are expected in activated sludge as can be seen from Table 5.1. This results from the considerable sludge flow of 10800 m<sup>3</sup> a day. Since about 99% of the activated sludge is returned to the biological tank, the most important amount of polycyclic musks keep turning in the waste water treatment plant. However this amount can be considered as trapped in the plant and not harming the environment, but if a material flux analysis is accomplished this flow has to be taken into account.

In order to compare expected primary sludge flows in the waste water treatment plant 'Mittleres Emmental' with actually measured values, fluxes measured in the waste water treatment plant Chevilly are reported in Table 5.1. Primary sludge flows (mg/cap/year) in Chevilly were measured and calculated by balancing the masses, to show how calculated and measured values vary. Since the waste water treatment plant in Chevilly is a one tank waste water treatment plant, fluxes in primary sludge can be calculated by subtracting the flows in treated waste water from waste water flows. Calculated and measured Celestolide, Traseolide, Galaxolide and Tonalide fluxes differ by less than 30% which lays in the range of acceptable differences. Phantolide flows are higher than the one expected and indicate that measured and expected values can differ up to 60%. It will be interesting to measure the concentrations in sludge sampled in the waste water treatment plant 'Mittleres Emmental'. Cashmeran concentrations in primary sludge were not measured in Chevilly and therefore no flow could be computed.

Expected flows in Chevilly lay within the same range as the flows expected in the waste water treatment plant 'Mittleres Emmental'. However the expected Celestolide flux in the waste water treatment plant 'Mittleres Emmental' is significantly higher than the flow calculated and found in Chevilly. Since the transfer coefficient for Celestolide between waste water and treated waste water is about the same in Chevilly and in the waste water treatment plant 'Mittleres Emmental', the difference results from the substantial higher inflow concentration measured in the waste water treatment plant 'Mittleres Emmental'. For all other compounds except Galaxolidone transfer coefficient lay in the same range for both waste water treatment plants as well but influent concentration in the waste water treatment plant 'Mittleres Emmental' is lower than in Chevilly, resulting in an absolute smaller difference between influent and effluent

concentrations, hence an absolute smaller amount is expected in primary sludge. Since influent rate (and effluent rate) per capita is about the same factor higher in 'Mittleres Emmental' than in Chevilly as the influent concentrations are lower, the expected flows in primary sludge are similar in both plants. It is evident that influent rate per capita is higher in the treatment plant 'Mittleres Emmental', because in Chevilly run-offs are not directed into the waste water treatment plant. For Galaxolidone flows differ due to considerable different transfer coefficients but the sum of Galaxolide and Galaxolidone is similar to the one computed in Chevilly.

Expected concentrations in primary sludge can be compared with concentrations measured in Chevilly Table 5.2. For Phantolide and Traseolide the differences are acceptable. Galaxolide and Galaxolidone as Tonalide concentrations are expected to be about the double higher in the waste water treatment plant 'Mittleres Emmental' than in Chevilly, which is still in the range of normal fluctuation. Celestolide concentrations are expected to be higher in the waste water treatment plant 'Mittleres Emmental' that is due to the higher waste water concentration.

Table 5.2: Expected sludge concentrations ( $\mu\text{g}/\text{kg dm}$ ) in activated and primary sludge calculated from mass balances and actual primary sludge concentration measured in Chevilly

	expected in activated sludge ( $\mu\text{g}/\text{kg dm}$ )	expected in primary sludge ( $\mu\text{g}/\text{kg dm}$ )	measured primary sludge Chevilly ( $\mu\text{g}/\text{kg dm}$ )	diff expected-Chevilly
Cashmeran	62	24	-	
Celestolide	671	442	47	-89%
Phantolide	273	152	202	33%
Traseolide	328	182	110	-40%
Galaxolide + Galaxolidone	19918	11324	4365	-61%
Tonalide	5977	3419	1461	-57%
Galaxolidone	25	-4	1225	-33737%

In the literature only few values for polycyclic musk concentrations in primary and activated sludge could be found. Results for primary and activated sludge are published in van de Plassche et al. (1997) and can be obtained from Appendix 1.6. They are with values for primary sludge of  $8600 \mu\text{g}/\text{kg}$  for Tonalide and  $13900 \mu\text{g}/\text{kg}$  for Galaxolide and for activated sludge of  $16000 \mu\text{g}/\text{kg}$  for Tonalide and  $27600 \mu\text{g}/\text{kg}$  for Galaxolide in the upper range than the one expected by calculations. It will be interesting to compare expected and measured values when analysis is accomplished.

## 5.2 PEC Values of Sludge

Van de Plassche et al. (1997) assessed transfer coefficients from waste water to primary sludge for Tonalide and Galaxolide as 0.81 and 0.83 respectively. From the amount of Galaxolide and Tonalide produced per year and capita and the sludge production per capita ( $71\text{g}/\text{d}/\text{cap}$ ) van de Plassche et al. compute the expected concentrations in primary sludge ( $150 \text{ mg}/\text{kg}$  dry matter (dm)). for Tonalide and  $389 \text{ mg}/\text{kg dm}$  for Galaxolide). The authors assume a reduction of 12% during digestion, which results in PEC for digested sludge of  $132 \text{ mg}/\text{kg dw}$  for Tonalide and  $336 \text{ mg}/\text{kg dw}$  for Galaxolide. The PEC concentrations in primary sludge are much higher as the ones measured and calculated above due to the reasons already accounted for when PEC concentrations in waste water were discussed.

Van de Plassche et al. calculated the transfer coefficients from the  $K_{ow}$  or  $K_{oc}$  values. Unfortunately it could not be assessed how this was done but from the know transfer coefficients concentrations for Tonalide and Galaxolide expected in the waste water treatment plant 'Mittleres Emmental' can be calculated by multiplying the flow in waste water per year with the transfer coefficient dividing it by the amount of primary sludge produced (personal communication: B. Bangerter  $973 \text{ t}/\text{dm}/\text{year}$ ). This results in an expected concentration in primary sludge of  $11259 \mu\text{g}/\text{kg dm}$  for Galaxolide and  $3520 \mu\text{g}/\text{kg dm}$  for Tonalide. These values are comparable with the values expected by balancing the masses within the plant, but they are higher than the values measured in Chevilly. Since for the other polycyclic musk compounds transfer coefficients were not calculated in the risk assessment by van de Plassche et al. (1997), no estimations can be accomplished for the remaining compounds.

### 5.3 Estimation of Polycyclic Musk Concentration from $K_{oc}$ or $K_{ow}$ Values

If it is assumed that the system in the primary or secondary settling tank or in the thickener corresponds to an organic carbon-water system in equilibrium, concentrations of polycyclic musks in sludge in the different tanks can be calculated from concentrations measured in water, the  $K_{oc}$  value and the fraction of organic carbon in sludge. The fraction of organic carbon in settled sewage sludge and activated sewage sludge was estimated 0.3 and 0.37 respectively by European Commission in the Technical Guidance Document for environmental risk assessments (cited by (Van de Plassche et al., 1997)).

$K_{oc}$  values for Cashmeran, Celestolide, Phantolide, Galaxolide and Tonalide can be found in the literature (Appendix 1.4). However it has to be considered that the values for Cashmeran, Celestolide and Phantolide were calculated from measured  $K_{ow}$  values of these compounds. No  $K_{oc}$  value could be found for Traseolide. Therefore it was calculated from the  $K_{ow}$  value ( $\log K_{ow}$  6.3) found in the literature, which is a computed value also. The  $K_{oc}$  for Traseolide was computed as described by Schwarzenbach et al. (1993) assuming a linear relationship between the  $\log K_{ow}$  values and the  $\log K_{oc}$  values of similar compounds, in this case polycyclic musks. This relationship was established by placing a regression line through the points originating from  $K_{ow}$  and  $K_{oc}$  values of the other polycyclic musk compounds found in the literature. The  $K_{oc}$  value for Traseolide was calculated by inserting the  $K_{ow}$  value in the equation found (Appendix 5.2).

No chemical properties for Galaxolidone have been described in the literature. Therefore  $K_{ow}$  as  $K_{oc}$  values had to be calculated. The  $K_{ow}$  value was estimated from the structurally related compound Galaxolide as described by Schwarzenbach et al. (1993) (Appendix 5.2). It is obvious that the  $K_{ow}$  value for Galaxolidone is lower than the  $K_{ow}$  of Galaxolide because Galaxolidone is more polar due to the ketone group. The  $K_{oc}$  value for Galaxolidone was derived from the linear relationship between  $K_{ow}$  and  $K_{oc}$  values as explained for Traseolide.

Table 5.3: Log  $K_{oc}$  values for polycyclic musk compounds

	log $K_{oc}$ (l/kg)
Cashmeran	3.75
Celestolide	4.47
Phantolide	4.80
Traseolide	5.21
Galaxolide	4.86
Tonalide	4.80
Galaxolidone	3.98

#### 5.3.1 Polycyclic Musk Concentrations in Primary Sludge

There are two tanks in the waste water treatment plant 'Mittleres Emmental' where a primary sludge-water system in equilibrium could be established: the primary settling tank and the thickener. In the primary settling tank musk concentrations in water correspond to the concentration in water after primary settling and  $K_{oc}$  values are given in Table 5.3. By the multiplication of the concentration in water with the  $K_{oc}$  values, expected concentrations of polycyclic musks in organic carbon can be calculated. Since an organic carbon fraction of 0.3 in primary sludge is assumed and dry weight was measured (Appendix 5.3), concentrations ( $\mu\text{g}/\text{kg}$  dw) of polycyclic musk in primary sludge can be computed. This concentrations can be compared with actual concentrations measured or can be used to calculate daily or annual fluxes. Results for daily calculation can be found in Appendix 5.4, means and measured values are outlined in Table 5.4. Fluxes can be calculated by multiplying the concentrations by the amount of primary sludge produced a year. Density of primary sludge was assumed to be 1.5  $\text{kg}/\text{l}$  as published by van de Plassche et al. (1997).

Analogically expected polycyclic musk concentrations can be calculated in the thickener, by multiplying the concentration found in supernatants with the  $K_{oc}$  values, multiplying with the organic carbon fraction (0.3) of primary sludge and dividing by the fraction of dry weight measured. Fluxes can be computed from the concentrations. Results can be found in Table 5.4 as well.

Table 5.4: Mean expected polycyclic musk concentrations in primary sludge, calculated from  $K_{oc}$  values and measured in the waste water treatment plant Chevilly

	primary sludge $\mu\text{g}/\text{kg dm}$			primary sludge $\text{mg}/\text{cap}/\text{year}$		
	primary settling tank	thickener	measured Chevilly	primary settling tank	thickener	measured Chevilly
Cashmeran	1'412	2'272	n.m	91	137	0
Celestolide	34'816	110'942	47	2'170	6'545	3
Phantolide	32'705	100'577	202	1'981	5'868	13
Traseolide	84'169	281'826	110	5'362	16'914	7
Galaxolide	2'371'746	7'660'697	3'140	147'888	459'364	207
Tonalide	668'014	2'248'849	1'461	42'055	134'870	96
Galaxolidone	3'862	10'551	1'225	242	640	80

Calculated and measured values differ three to four orders of magnitude and it cannot be claimed that these differences origin from the fact that the concentrations were not measured in the same waste water treatment plant only. This indicates that the system established in the thickener and the primary settling tank can not be compared with an organic carbon system established in a laboratory.

The smallest differences between the fluxes estimated and measured is found for Galaxolidone. Concentrations as  $K_{oc}$  values are low for this compound, which results in low flows expected. However these values might differ considerably from actual concentrations because it is possible that Galaxolidone is produced from the degradation of Galaxolide.

The differences between concentrations calculated from  $K_{oc}$  values and measured concentrations are much higher than between concentrations calculated by balancing the masses and actual measured concentrations. Reasons for the high concentrations expected if calculated by  $K_{oc}$  values could be that much of the suspended matter, that may adsorb a high percentage of polycyclic musk does not settle in thickener or in the primary settling tank and therefore this amount is measured in water or that adsorption to surfaces influences the system considerably. It is deduced that, if only water concentrations are measured in a study, it is more appropriate to calculate musk concentrations by balancing the masses instead of calculating the concentrations in sludge by the  $K_{oc}$  values or if  $K_{oc}$  values are used transfer coefficients have to be calculated thereof as accomplished by van de Plassche et al. (1997).

### 5.3.2 Polycyclic Musk Concentrations in Activated Sludge

It can be assumed that as in the primary settling tank, in the secondary settling tank polycyclic musks partition between water and organic carbon according to the  $K_{oc}$  value. From the concentration of polycyclic musks measured in treated waste water and the  $K_{oc}$  value, the expected concentration in organic carbon can be calculated. By multiplying the result with the carbon fraction in activated sludge (0.37) and with the flow ( $\text{m}^3/\text{day}$ ) of activated sludge (Table 3.1), which was converted to  $\text{l}/\text{day}$  with the density of 1.3 (van de Plassche et al. 1997), daily flows can be estimated. Mean values are given in Table 5.5 and daily values are reported in (Appendix 5.4).

Table 5.5: Mean expected polycyclic musk concentrations ( $\mu\text{g}/\text{kg dm}$ ) and flows ( $\text{mg}/\text{cap}/\text{year}$ ) in activated sludge, calculated from  $K_{oc}$  values and by mass balancing the system

	activated sludge $\mu\text{g}/\text{kg dm}$		activated sludge $\text{mg}/\text{cap}/\text{year}$	
	expected	calculated by $K_{oc}$	expected	calculated by mass balancing
Cashmeran		7'918	12'511	98
Celestolide	193'193		300'617	1057
Phantolide	181'323		283'191	430
Traseolide	472'308		739'805	516
Galaxolide	13'270'467		20'981'379	31368
Tonalide	3'738'403		5'904'339	9414
Galaxolidone	21'586		33'460	40

Flows calculated from the  $K_{oc}$  values differ considerably from the expected concentrations obtained by balancing the masses as for primary sludge. Unfortunately polycyclic musk concentrations have never been measured in activated sludge and therefore calculated values cannot be compared with actually measured values. However due to the impressively high values expected, it can be stated that it is more adequate to calculate concentrations from mass balance than by the  $K_{oc}$  values as found for primary sludge before. Possible reasons for these findings are the same as named above, suspended matter that adsorbs polycyclic musks, which is analysed in water, and a system that does not correspond to a laboratory organic carbon-water system.

The storage tank can also be considered as a system at equilibrium, so that from concentrations of polycyclic musks in supernatants, musk concentrations in sludge could be estimated. However since the results obtained for the primary settling and the secondary settling tank do not seem to represent concentrations found in actual waste water treatment plants, calculations have not been accomplished for the storage tank.

Conclusively it can be stated that concentrations in sludge can be estimated by mass balancing the system, but by assuming a simple water-organic carbon system at equilibrium only and applying  $K_{oc}$  values, it is impossible to predict polycyclic musk concentration in sludge. However there has to be a system to deduce transfer coefficients between water and sludge from  $K_{oc}$  values as accomplished by van de Plassche et al. (1997), but from the publication it could not be deduced how it was done.

## 6. Conclusion

A method to analyse polycyclic musk compounds in waste water was established in the Laboratory for Environmental Chemistry and Ecotoxicology (CECOTOX) at the Swiss Federal Institute of Technology in Lausanne. It was found that polycyclic musk concentrations in waste water decrease significantly during the waste water treatment process. Transfer coefficients between waste water and treated waste water have been computed and are in accordance with data found in the literature. It is assumed that most of the musks removed, accumulate in sludge, since these compounds are only poorly biodegradable. Nevertheless this hypothesis has to be verified by analysing sludge but since the analysis method is different, it could not be established fully in the time being. This will be done in a second phase of this project and result in a complete material flux analysis with established transfer coefficients. Based on these transfer coefficients it will be possible to monitor polycyclic musk flows from sludge analysis and estimate inflow as outflow concentrations in a waste water treatment plant.

As a basic principle polycyclic musk concentrations measured in water samples taken from a representative waste water treatment plant are comparable to the data found in the literature and within other SEA projects, which shows a lower contamination of waste water by polycyclic musk compounds than expected by the Predicted Environmental Concentration. PEC values were determined for Tonalide and Galaxolide from the amount of polycyclic musks produced in Europe and even if Galaxolidone as a metabolite is added to the Galaxolide concentration predicted concentrations are by a factor 40 higher than measured concentrations for both compounds. According to the estimation methods used in the EU-TGD risk assessment PEC values for other polycyclic musk compounds have been computed also. These concentrations are higher as well than the concentrations measured. Further research is required to explain the differences found. It is suggested to start with accounting better for the production data, since the sources of these values are difficult to obtain and since measured waste water concentrations were confirmed in other studies. There might also be another way of environmental contamination, or polycyclic musks are degradable considerably and therefore not 100% of the amount produced reaches the waste water treatment plant. The assessment of biodegradability of polycyclic musks would be another research subject.

Due to daily sample analysis, it was found that musk concentrations in waste water vary on different days of the week. For most of the compounds peak concentrations were measured on Monday. In order to assess the effect of peoples washing and personal care behaviour on the polycyclic musk concentrations in waste water, further analysis would be required including a sociological survey and an analysis of detergents and cosmetic products, since polycyclic musk content of these products have not been assessed fully. This would imply a considerable research project and since a threat of the environment by polycyclic musks is not obvious for the time being, expenses demanded have to be questioned. However the daily variation might also be explained by statistical variation.

Sludge concentrations have been calculated by balancing masses in the waste water treatment plant and were compared with measured concentrations in another plant of the SEA network. It was found that the calculated and expected concentrations lay in the same range. If sludge concentrations are estimated from  $K_{oc}$  values, the expected concentrations are much higher than the measured. This leads to the conclusion, that sludge concentrations are better computed by mass balancing than by  $K_{oc}$  values.

This report contributes to the discussion on polycyclic musks and in general to lipophilic compounds in the environment. As seen for other compounds chemicals synthesised by humans can pose a considerable threat to the environment and therefore need to be watched closely.



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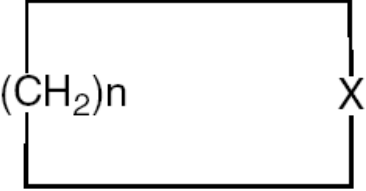
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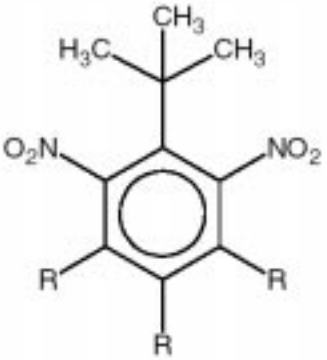
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## Appendix 1.1 Chemical structure of Musk Compounds

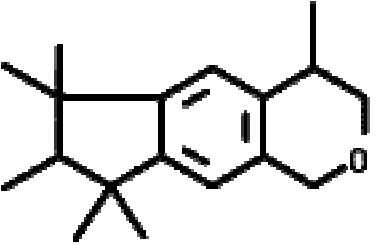
### Macrocyclic Musks

	<p>Macrocyclic musks include natural musks and synthetic musks that are deduced from this structure. X stands for a functional or a combination of functional groups. Compounds with a musky smell exist of 15-17 C-atoms, as explained by Wiegel et al. (2000). Rebmann (1997) accounts that the group of macrocyclic musks contains besides monocyclic bicyclic systems as well.</p>
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### Nitro Musks

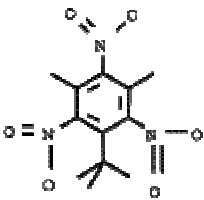
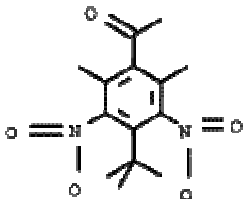
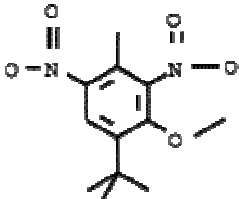
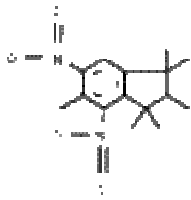
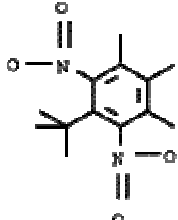
	<p>Nitro Musks are closely related to each other. They consist of a double or threefold substituted benzene ring with a tertiary butyl group. R-substitutes are alkyl-, acetyl- or methoxygroups.</p>
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### Polycyclic Musks

	<p>Polycyclic musks are bi- or tricyclic compounds, normally with an aromatic ring. Their structures differ considerably as can be seen in Appendix 1.3 where the most important polycyclic musks are reproduced. As an example the chemical structure of Galaxolide is given here</p>
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## Appendix 1.2 Chemical Structure of Nitro Musks

Table 1.2.1: Chemical structure of nitro musks

Common Name	Chemical Name		CAS-Number	Chemical Formula	Molecular Weight
Musk Xylol	1-tert-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene		81-15-2	C <sub>12</sub> H <sub>15</sub> N <sub>3</sub> O <sub>6</sub>	297,27
Musk Keton	4-tert-Butyl-2,6-dimethyl-3,5-dinitroacetophenone		81-14-1	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub>	294,31
Musk Ambrette	2,6-Dinitro-3-methoxy-4-tert-butyltoluol		83-66-9	C <sub>12</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	268,27
Muskene	1,1,3,3,5-Pentamethyl-4,6-dinitroindane		116-66-5	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	278,31
Musk Tibetene	1-tert-Butyl-3,4,5-trimethyl-2,6-dinitrobenzene		145-39-1	C <sub>13</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	266,30



## Appendix 1.3 Chemical Structure of Polycyclic Musks

Table 1.3.1: Chemical structure of polycyclic musks

Common Name	Chemical Name		CAS-Number	Chemical Formula	Molecular Weight
Galaxolide Abbalide	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyran (HHCB)		1222-05-5	C <sub>18</sub> H <sub>26</sub> O	258,40
Tonalide Fixolide Astralide	6-Acetyl-1,1,2,4,4,7-hexamethyltetralin (AHTN)		1506-02-1	C <sub>18</sub> H <sub>26</sub> O	258,40
Celestolide Crysolide	4-Acetyl-1,1-dimethyl-6-tert-butyl-dihydroindene (ADBI)		13171-00-1	C <sub>17</sub> H <sub>24</sub> O	244,37
Phantolide	6-Acetyl-1,1,2,3,3,5-hexamethylindane (AHMI) 6-Acetyl-1,1,2,3,3,5-hexamethyldihydroinden (AHDI)		15323-35-0	C <sub>17</sub> H <sub>24</sub> O	244,37
Traseolide	5-Acetyl-3-isopropyl-1,1,2,6-tetramethylindane (AITI) 5-Acetyl-1,1,2,6-tetramethyl-3-isopropyl-dihydroinden (ATII)		68140-48-7	C <sub>18</sub> H <sub>26</sub> O	258,40
Cashmeran	1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4Hinden-4-one (DPMI)		33704-61-9	C <sub>14</sub> H <sub>22</sub> O	206,32
Galaxolidone	Oxidation Product of Galaxolide			C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	272,39

## Appendix 1.4 Chemical Properties of Polycyclic Musks

Table 1.4.1: Chemical properties of polycyclic musk compounds (for references see end of Appendix 1.6)

	<b>Galaxolide</b> HHBC	<b>Tonalide</b> AHTN	<b>Celestolide</b> ADBI	<b>Phantolide</b> AHMI	<b>Traseolide</b> AITI	<b>Cashmeran</b> DPMI	<b>Galaxolidone</b>
CAS-Number	1222-05-5	1506-02-1	13171-00-1	15323-35-0	68140-48-7	33704-61-9	
Chemical Formula	C <sub>18</sub> H <sub>26</sub> O	C <sub>18</sub> H <sub>26</sub> O	C <sub>17</sub> H <sub>24</sub> O	C <sub>17</sub> H <sub>24</sub> O	C <sub>18</sub> H <sub>26</sub> O	C <sub>14</sub> H <sub>22</sub> O	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>
mol. weight	258.4	258.4	244.38	244.38	258.4	206.32	272.39
physical state	viscous liquid [2]	solid [2]	solid	solid	viscous liquid	solid	solid
melting point (°C) (measured)	inapplicable [2]	54.5 [2]					
vapour pressure (Pa) (measured)	0.0727 [2]	0.0682 [2]					
<b>Solubility in Water</b>							
C <sub>w</sub> (mg/l) (measured)	1.75 [2]	1.25 [2]					
C <sub>w</sub> (mg/l) (measured)	1.65 [17]	1.22 [17]					
C <sub>w</sub> (mg/l) (calculated)	0.19 Balk 1998 in [12]	0.36 Balk 1998 in [12]	0.22 Balk 1998 in [12]	0.25 Balk 1998 in [12]	0.09 Balk 1998 in [12]		
<b>K<sub>H</sub> values</b>							
K <sub>H</sub> (Pa·m <sup>3</sup> /mol)	11.3 [2]	12.5 [2]					
<b>K<sub>ow</sub> values</b>							
log K <sub>OW</sub> (measured)	5.7 [2]	5.9 [2]					
log K <sub>OW</sub> (measured)	5.88 Eschke, 1994 in [19]	5.77 Eschke, 1994 in [19]	5.4 [12]	5.8 Eschke 1999 in [14]		4.5 Eschke 1999 in [14]	
log K <sub>OW</sub> (calculated)	6.3 Balk 1998 in [12]	6.4 Balk 1998 in [12]	5.9 Balk 1998 in [12]	5.85 Balk 1998 in [12]	6.3 Balk 1998 in [12]		
log K <sub>OW</sub> (calculated, fragmentation method)			5.4 Gatermann et al. 1998 [11]	5.9 Gatermann et al. 1998 [11]	6.3 Gatermann et al. 1998 [11]	5.9 Gatermann et al. 1998 [11]	
<b>K<sub>oc</sub> values</b>							
log K <sub>OC</sub> (l/kg) (measured)	4.86 [17]	4.8 [17]					
log K <sub>OC</sub> (l/kg) (calculated from K <sub>ow</sub> )	4.88 [17]	4.71 [17]	4.47 [19]	4.8 [19]		3.75 [19]	

	Galaxolide HHBC	Tonalide AHTN	Celestolide ADBI	Phantolide AHMI	Traseolide AITI	Cashmeran DPMI	Galaxolidone
<b>Half life times</b>							
atmospheric lifetime due to the reaction with OH	5.3 h [1]						
biodegradation rate	0 hr <sup>-1</sup> [2]	0 hr <sup>-1</sup> [2]					
<b>Proportion of polycyclic musks in environmental samples</b>							
Proportion in surface water samples [19]	16	10					
Proportion in sediments originating from suspended matter [19]	20	10	0.3				
Water of the River Elbe [20]	20	10					
treated waste water [5]	15	20	1				
Proportion in surface water in Berlin [8]	20	10	1				
waste water Chevilly [3]	210	45	2.5	6	5	1	13
water after primary settling Konolfingen [3]	120	43	4	4.5	4	1	19
treated waste water Chevilly [3]	122	35	2	9	3	1	128
treated waste water Konolfingen [3]	35	13	2	2	2	1	47

## Appendix 1.5 Concentrations of Polycyclic Musks in Waste Water, Water after Primary Settling and Treated Waste Water (Compilation from the Literature)

### Galaxolide (HHBC)

Table 1.5.1: Galaxolide concentrations in waste water, water after primary settling and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					water after primary settling (ng/l)		treated waste water (ng/l)				
	med.	mean	max	remarks	n	mean	remarks	med.	mean	max	remarks	n
Germany, Ruhr Eschke et al., 1994 in [2]	1500*			settled infl.	21			1900			90% 2900	30
Germany, Ruhr [4]		1460		500-2900	7				1090		600-2000	7
Germany, Ruhr Eschke et al., 1995 in [17] <sup>1</sup>								1200			600-2500 90% 2400	21
Germany, Ruhr Eschke pers. communication. to the RIVM, 1996 in [19]				10000-30000	7						10000-30000	7
Germany, Berlin [6]								6650	6850	13300	SD 2640 90% 10800	30
WWTP Schönerlinde (Berlin) [8]									10800			3
WWTP Falkenberg (Berlin) [8]									6300			3
WWTP Münchhofen [8]									10100			3
Schleswig-Holzstein [7]									4200 3700		two random samples	1
Netherlands Rijs and Schläpfer, 1999 in [2]	6400		14500	raw infl.	9			1400		1600		-
Netherlands [18]									321		162-629	4
Sweden [13]											1000-6000	3
Europe [16]		9710		SD 5090								
USA activated sludge WWT [15]		13700		SD 1500		7760	SD 1600		1170		SD 100	
USA trickling filter WWT [15]		9810				6660			1630			
USA [16]		16600		SD 10400	12				2053		SD 1314	4
Switzerland 17 STEP SAFEL 1998 in [12]								2300			1100-5600	17
Switzerland 6 STP in BL [10]								3000	2983			6
Switzerland, Chevilly [3]		6901		SD 1498 5394-9018	7				857		SD 127 729-1078	7
Switzerland, Konolfingen [3]						1682		1	255			1
Switzerland 'Mittleres Emmental' (this study)	4532	4507	6937	SD 1404 2339-6937	7	3505	SD 871 4944-2434	7	780	1052	SD 182 582-1052	7
PEC [17]		160000							25000			

\* unreliable results according to the author, more likely 10-30 µg/l

<sup>1</sup> includes data from Eschke et al. 1994, second line

## Tonalide (AHTN)

Table 1.5.2: Tonalide concentrations in waste water, water after primary settling and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					water after primary settling (ng/l)		treated waste water (ng/l)				
	med.	mean	max	remarks	n	mean	remarks	med.	mean	max	remarks	n
Germany, Ruhr Eschke et al., 1994 in [2]		2200*		settled infl.	21			1800			90% 3000	30
Germany, Ruhr [4]		2240		800-4400	7				1400		800-2400	7
Germany, Ruhr Eschke et al., 1995 in [17] <sup>1</sup>								1600			800-3100 90% 3000	21
KA Berlin Herberer et al 1999. in [19]											800-3100	3
Germany, Berlin [6]								2160	2240	4310	SD 860 90% 3360	30
WWTP Schönerlinde (Berlin) [8]									5800			3
WWTP Falkenberg (Berlin) [8]									1950			3
WWTP Münchehofe [8]									5800			3
sewage effluent Schleswig-Holzstein [7]									1900 1700		two random samples	1
Netherlands Rijs and Schläpfer, 1999 in [2]	4000		8700	raw infl.	9			<d.l.		770		8
Netherlands [18]									277		110-420	4
Europe [16]		5970		SD 3880					1440			1
USA activated sludge WWT [15]		10700		SD 619		6990	SD 1340		1180		SD 74	
USA trickling filter WWT [15]		10000				7030			1660			
USA [16]		12500		SD 7350	12				1326		SD 270	4
Switzerland 17 STEP SAFEL 1998 in [12]								1400			90% 2000 500-2400	17
Switzerland 6 STP in BL [10]								2037	1960			6
Switzerland, Chevilly [3]		1518		SD 379 1241-2280					247		SD 67 181-365	7
Switzerland, Konolfingen [3]						600	n=1		94			1
Switzerland 'Mittleres Emmental' (this study)	1400	1454	2040	SD 290 2040-1150	7	1132	SD 242 841-1486	304	328	513	SD 117 192-513	7
<b>PEC [17]</b>		66000							11000			

\* unreliable results according to the author, more likely 10-30 µg/l

<sup>1</sup> includes data from previous line

### Celestolide (ADBI)

Table 1.5.3: Celestolide concentrations in water and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					treated waste water (ng/l)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n
3 WWTP area Ruhr Eschke et al., 1995 in [12]						80			90% 100	15
Germany, Ruhr [4]		80		40-140			60		40-70	
Germany, Berlin [6]						120	110	210	SD 40 90% 170	30 (100)
WWTP Schönerlinde (Berlin) [8]							410			3
WWTP Falkenberg (Berlin) [8]							430			3
WWTP Münchehofe [8]							110			3
Rijs 1998 in Balk 1998 in [12]	nq.		290		9					
Switzerland 17 STEP SAFEL 1998 in [12]						nq (<100)			90 % 124 <100-160	17
Switzerland 6 STP in BL [10]						75	83			6
Switzerland, Chevilly [3]		84		SD 21 53-114			16		SD 3 12-21	7
Switzerland, Konolfingen [3]		58.3		sett. influent	1		13.2			1
Switzerland 'Mittleres Emmental' (this study)	154	179	329	SD 80 91-329	7	27	30	43	SD 8 21-43	7

### Phantolide (AHMI)

Table 1.5.4: Phantolide concentration in waste water and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					treated waste water (ng/l)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n
Rijs 1998 in Balk 1998 in [12]	nq.		700							
Germany, Berlin [6]						230	270	580	SD 130 90% 360	12 (100)
Switzerland 6 STP in BL [10]						100	104			6
Switzerland, Chevilly [3]		195		SD 85 101-337			65		SD 14 50-88	7
Switzerland, Konolfingen [3]		63.5		sett. influent	1		15.6			1
Switzerland 'Mittleres Emmental' (this study)	55	67	112	SD 30 31-112	7	17	17	22	SD 3 12-22	7

### Traseolide (AITI)

Table 1.5.5: Traseolide concentration in waste water and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					treated waste water (ng/l)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n
Rijs 1998 in Balk 1998 in [12]	nq.		570							
Germany, Berlin [6]						260	210	700	SD 20 90% 620	25 (96)
Switzerland 6 STP in BL [10]						40	43			6
Switzerland, Chevilly [3]		169		SD 79 87-321	7		19		SD 6 13-30	
Switzerland, Konolfingen [3]		57		sett. influent	1		14.3			1
Switzerland 'Mittleres Emmental' (this study)	67	71	104	SD 15 65-104	7	10	10	15	SD 2 9-15	7

### Cashmeran (DMPI)

Table 1.5.6: Celestolide concentration in waste water and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					treated waste water (ng/l)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n
Switzerland 6 STP in BL [10]									<500	
Switzerland, Chevilly [3]		33		SD 10 21-51	7		7		SD 1 5.1-8.1	7
Switzerland, Konolfingen [3]		13.5		sett. influent	1		7.2			1
Switzerland 'Mittleres Emmental' (this study)	27	26	37	SD 8 13-37	7	17	18	25	SD 5 13-25	7

### Galaxolidone

Table 1.5.7: Galaxolidone concentration in waste water and treated waste water (ng/l) (for references see end of Appendix 1.6)

	waste water (ng/l)					treated waste water (ng/l)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n
Switzerland, Chevilly [3]		426		SD 40 385-503	7		899	SD 223 682-1194		7
Switzerland, Konolfingen [3]		276		sett. influent	1		344			1
Switzerland 'Mittleres Emmental' (this study)	37	37	69	SD 20 0-69	7	39	39	48	SD 7 30-48	7

## Appendix 1.6 Concentrations of Polycyclic Musks in Sewage Sludge

### Galaxolide (HHBC)

Table 1.6.1: Galaxolide concentration in different type of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm.}$ )					activated sludge ( $\mu\text{g}/\text{kg dm.}$ )					sludge ( $\mu\text{g}/\text{kg dm.}$ )				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med	mean	max	remarks	n
Switzerland [9] fresh sewage sludge											3896	4853	12157	90% 7853	12
16 ARA Switzerland [3]												20336		11571- 31397	21
Chevilly [3]		3140		2313- 3846	7										
Germany (2 STPsWWTPs) Sauer et al. 1997 in [2]							1400		100-5200	17		8870		4300- 13400	2
sewer slime urban/industrial area Sauer et al. 1997 in [2]												15500		9100- 21800	2
Hessen, Germany Fooker'97 in Rimkus et al. 1998a in [12]												16930		11886- 21626	9
Hessen, Germany Fooker et al. 1999 in [19]														6704- 22288	
KA Berlin Herberer et al 1999. in [19]														6-10	3
Sludge Ruhr Eschke, pers. comm. in [17]														45000	1
Sludge Schleswig-Holzstein [7]											4800			wet weight!	1
Netherlands (digested sludge) Blok 1998 in [12],											23000			SD 7200 11000- 31000	8
NL Rijs and Schläpfer, 1999 [2]	13500			6000- 17000	8	9700			0-21000	7					
NL digested, Rijs and Schläpfer, 1999 [2]												20000		19000- 21000	2
NL Block, 1997 in [17]		13900		5400- 27000	11		27900		4400- 63000	12	23000 dig.sludge	19900 dig.sludge		9000- 31000	13
PEC [17]												336'000			



**Tonalide (AHTN)**Table 1.6.2: Tonalide concentration in different types of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm}$ .)					activated sludge ( $\mu\text{g}/\text{kg dm}$ .)					sludge ( $\mu\text{g}/\text{kg dm}$ .)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med	mean	max	remarks	n
Switzerland [9] fresh sewage sludge											1321	1537	4161	90% 1964	12
16 ARA Switzerland [3]												7262		3398-11203	21
Chevilly [3]		1461		1062-1753	7										
Germany (2 WWTPs) Sauer et al. 1997 in [2]							2100		100-8900	17		8300		4000-12600	2
sewer slime urban/industrial area Sauer et al. 1997 in [2]												23100		9500-36700	2
Hessen, Germany Fooker'97 in Rimkus et al. 1998a in [12]												15450		11992-20107	9
Hessen, Germany Fooker et al. 1999 in [19]														5752-18436	9
Sludge Ruhr Eschke, pers. comm. in [17]														42000	1
sludge Schleswig-Holzstein [7]											2000			wet weight!!	1
Netherlands (digested sludge) Blok 1998 in [12],											14800			SD 5000 6200-21000	8
NL Rijs and Schläpfer, 1999 [2]	8200			3700-11700	8	5300			0-13500	7					
NL digested Rijs and Schläpfer, 1999 [2]												12000		11000-13000	2
NL Block, 1997 in [17]		8300		3300-14000	11		16000		2300-34000	12	16000	13500		4900-22000	13
<b>PEC [17]</b>												132000			

### Celestolide (ADBI)

Table 1.6.3: Celestolide concentration in different type of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm}$ .)					activated sludge ( $\mu\text{g}/\text{kg dm}$ .)					sludge ( $\mu\text{g}/\text{kg dm}$ .)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med.	mean	max	remarks	n
Switzerland [9] fresh sewage sludge											113	130		41-330 90% 234	12
16 ARA Switzerland [3]												427		139-1087	21
Chevilly [3]		47		37-57	7										
Germany 2 STP Sauer et al. 1997 in [12]												200		120-290	2
Netherlands 4 WWTPs digested sludge Blok 1998 in [12]											1100			<1000- 1800	8

### Phantolide (AHMI)

Table 1.6.4: Phantolide concentration in different type of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm}$ .)					activated sludge ( $\mu\text{g}/\text{kg dm}$ .)					sludge ( $\mu\text{g}/\text{kg dm}$ .)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med	mean	max	remarks	n
Switzerland [9] fresh sewage sludge											203	303		65-842 90% 781	12
16 ARA Switzerland [3]												811		407-1663	21
Chevilly [3]							202		160- 233	7					
Netherlands 4 WWTPs digested sludge Blok 1998 in [12]											1250			<1000- 2200	

### Traseolide (AITI)

Table 1.6.5: Traseolide concentration in different type of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm}$ .)					activated sludge ( $\mu\text{g}/\text{kg dm}$ .)					sludge ( $\mu\text{g}/\text{kg dm}$ .)				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med	mean	max	remarks	n
Switzerland [9] fresh sewage sludge											nq			nq	
16 ARA Switzerland [3]												672		256-1010	21
Chevilly [3]							110		77-137	7					
Netherlands 4 WWTPs digested sludge Blok 1998 in [12]											1950	2200		SD 885 1200- 3600	

### Cashmeran (DMPI)

Table 1.6.6: Cashmeran concentration in different types of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm}$ )					activated sludge ( $\mu\text{g}/\text{kg dm}$ )					sludge ( $\mu\text{g}/\text{kg dm}$ )				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med.	mean	max	remarks	n
Switzerland [9] fresh sewage sludge											68	93		38-332 90% 142	12
Chevilly [3]						nd	nd	nd							

### Galaxolidone

Table 1.6.7: Galaxolidone concentration in different types of sludge ( $\mu\text{g}/\text{kg dm}$ ) (for references see end of Appendix 1.6)

	primary sludge ( $\mu\text{g}/\text{kg dm}$ )					activated sludge ( $\mu\text{g}/\text{kg dm}$ )					sludge ( $\mu\text{g}/\text{kg dm}$ )				
	med.	mean	max	remarks	n	med.	mean	max	remarks	n	med.	mean	max	remarks	n
16 ARA Switzerland [3]												1781		754-3319	21
Chevilly [3]							1225		1110-1366	7					

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## Appendix 1.7 Toxicity Data for Some Polycyclic Musks

Table 1.7.1: Toxicity data for some polycyclic musks

	Galaxolide (HHBC)	Tonalide (AHTN)	Phantolide (AHMI)	Traseolide (AITI)
Algae <sup>1</sup> (mg/l) ( <i>Pseudokichneriella subcapita</i> )	72h NOEC 0.201 EC50 0.732	72h NOEC 0.276		
Daphnia <sup>1</sup> (mg/l) ( <i>Daphnia magna</i> )	21d NOEC 0.111 EC50 0.282	21d NOEC 0.196 EC 50 0.244	EC50 48h 0.33 <sup>2</sup>	EC50 48h 0.42 <sup>2</sup>
fish (mg/l) <sup>1</sup> <sup>a</sup> <i>Brachydanio rerio</i> <sup>b</sup> <i>Pimephales promelas</i>	<sup>a</sup> 36d NOEC 0.093 LC50 0.452 <sup>b</sup> 36d NOEC 0.068 LC50 > 0.14	<sup>a</sup> 36d NOEC 0.089 LC50 0.314 <sup>b</sup> 36d NOEC 0.035 LC50 0.100		
PNEC water <sup>1</sup> (mg/l)	0.0068	0.0035		
earthworm <sup>1</sup> (mg/kg) ( <i>Eisenia foetida</i> )	8 wk-NOEC 45	8 wk NOEC 105		
springtail <sup>1</sup> (mg/kg) ( <i>Folsomia candida</i> )	4 wk NOEC 45	4 wk NOEC 45		
PNEC soil <sup>1</sup> (mg/kg dw)	0.32	0.32		
rats (mammals) <sup>1</sup> (mg/kg bw)	150	15		
PNEC pred <sup>1</sup> (mg/kg food)	100	10		

<sup>1</sup> Source: (Van de Plassche et al., 1997)<sup>2</sup> Source: (OSPAR Commission, 2000)

Table 1.7.2: Bioconcentration factors of polycyclic musks on a wet basis

	HHCB	AHTN	ADBI	AHMI	DPMI
BCF (mol/kg wet weight (lipid %))	mussel (1.4%) 620 fish 624-1624	mussel (1.4%) 560 fish 597-1320	fish (5%) 670	fish (5%) 1670	fish (5%) 84

Source: Geyer et al. 2000

### Appendix 3.1 Location of the Waste Water Treatment Plant 'Mittleres Emmental' in Hasle (BE), Switzerland



Figure 3.1.1

Location of the waste water treatment plant 'Mittleres Emmental' in Hasle (BE), Switzerland

## Appendix 3.2 Characteristics of the Waste Water Treatment Plant 'Mittleres Emmental'

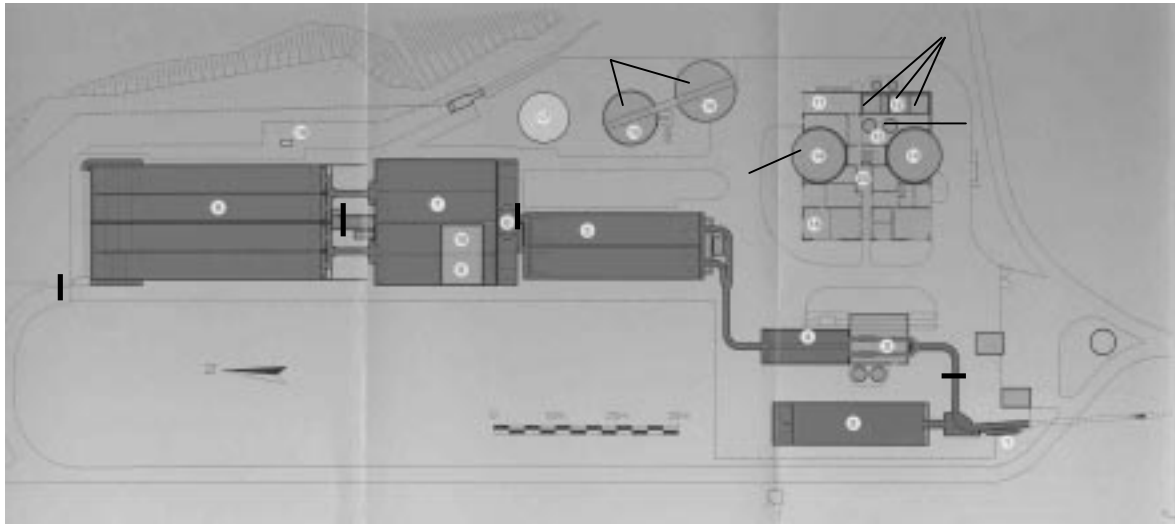


Figure 3.2.1

Map of the waste water treatment plant 'Mittleres Emmental'

<b>Sewage Treatment</b>	<b>Volume</b>	<b>Sludge Treatment</b>	<b>Volume</b>
1. gravel trap		11. sludge screening	
2. storm tank	640 m <sup>3</sup>	12. thickener	3*110 m <sup>3</sup>
3. screen (16mm)		13. disinfection plant	2*60 m <sup>3</sup> /d
4. aerated sandtrap	2*180 m <sup>3</sup>	14. digester	2*1200 m <sup>3</sup>
5. primary settling tank	2*500 m <sup>3</sup>	15. sludge storage tank 1	800 m <sup>3</sup>
6. selectors	2*100 m <sup>3</sup>	16. sludge storage tank 2	1500 m <sup>3</sup>
7. aerated tank	4*650 m <sup>3</sup>	17. gas tank	500 m <sup>3</sup>
8. secondary settling tank	4*780 m <sup>3</sup>	18. block heat and power plant	
9. fan station		19. gas flare	
10. phosphor elimination		20. premises	

- a waste water sampling station
- b sampling station for water after primary settling
- c sampling station for treated waste water
- d sampling station for activated sludge
- e sampling station for primary sludge and supernatants from primary settling tanks
- f sampling station for sludge and supernatants from storage tanks
- g sampling station for sludge from digester
- h sampling station for sludge from disinfection



### Appendix 3.3 Catchment's Area of the Waste Water Treatment Plant 'Mittleres Emmental'

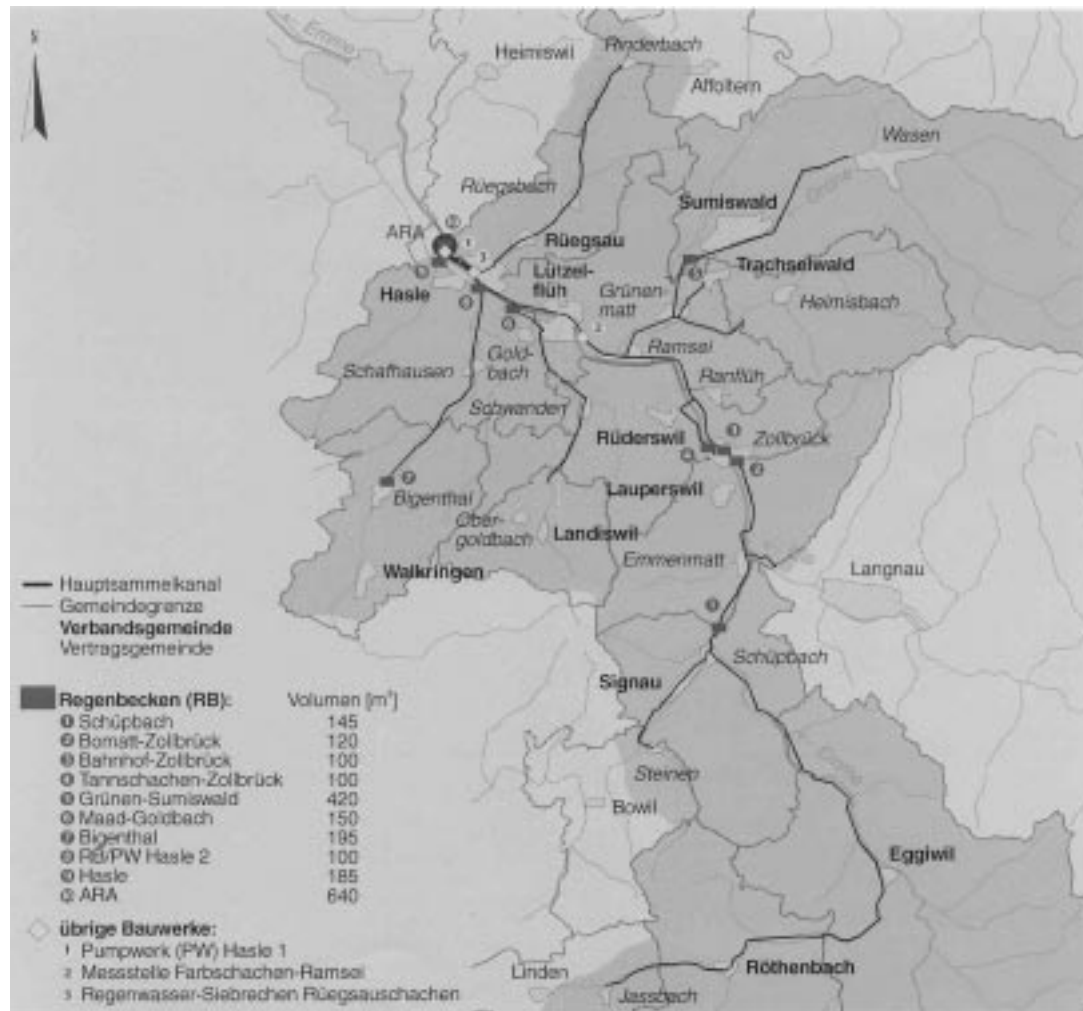


Figure 3.3.1

Catchment's area of the waste water treatment plant 'Mittleres Emmental'

## Appendix 3.4 Progression of the Sampling

### Waste Water: Sampler type Bühler PB-MOS (P 33 / P34 / P35)

Day	Date	Start time	start of im-pulsion counter	# of sam-ples taken	remarks	Sample number
Tuesday	9.4.2002	7h20		264	Sampling with PVC tube	269
Wednesday	10.4.2002	7h36	506796	-	interrupted once, sampling with PCV tube	276
Thursday	11.4.2002	8h32	507601	251		285
Friday	12.4.2002	7h31	508345	262		292
Saturday	13.4.2002	6h40	509125	171	height adjusted to from 25mm to 22 mm sampler might have stopped at 18h24.	299
Sunday	14.4.2002	7h14 <b>p33</b> / *9h04 <b>p34</b>	510956 / 511120	52 /	interrupted at about 9h30	n.a
Monday	15.4.2002	7h35 16h50	512434 512806	123 63	interrupted at 21h47; error message: Störung Füllstandsmelder	n.a
Sunday	21.4.2002	7h20 with <b>p33</b> break down 13h30 Restart 14h05 break down 14h08 Restart 14h22 break down 21h08 Restart 21h40 break down 21h51 Restart 22h22 with <b>p34</b> break down 22h30 Restart 23h05 break down 0h28  Restart 1h07	517466  517674  517684  517922  517945 517950 517969	63 2  74  1  43 Total 183	height adjusted to 30 mm        The last 20 samples (about 1dl), were sampled from red coloured water (slaughter house)	307
Monday	22.4.2002	7h38 with <b>p 35</b> break down 9h31 Restart 10h22 break down après 12h00 Restart 13h30	518178	15  9	height adjusted to 32 mm, between 21h00 and 23h00 inflow of red coloured water (dye works?)	314
Tuesday	23.4.2002	sampling end 7h23	518759	139		
			Total	163		

\*Reason to change programme: flow about 300 m<sup>3</sup>/sec

n.a: not analysed

**Programme of waste water sampler:**

Programme 33

Impulsteiler: 3 (every 3rd impulse (every 30 m<sup>3</sup>) a sample is taken)

Samples per flask: 290

Quantity of sample per impulsion: 75 ml, height about 28 mm

Programme 34

Impulsteiler: 5 (every 5th impulse (every 30 m<sup>3</sup>) a sample is taken)

Samples per flask: 400

Quantity of sample per impulsion: 50 ml, height about 21 mm, 100 ml 35 mm

Programme 35

Impulsteiler: 4 (every 4th impulse (every 40 m<sup>3</sup>) a sample is taken)

Samples per flask: 290

Quantity of sample per impulsion: 85 ml; height about 32 mm

**Effluent primary settling tank (Sampler type: IBUK-KS 350, IBUK PNV-MC)**

Sampling proportional to the flow, every 2 impulsions (1 impulsion = 10 m<sup>3</sup>) a sample is taken, Quantity sampled per impulsion: 75 ml,

Day	Date	Start time	remarks	sample number
Tuesday	9.4.2002	7h30		270
Wednesday	10.4.2002	7h47		277
Thursday	11.4.2002	8h50		286
Friday	12.4.2002	7h55		293
Saturday	13.4.2002	6h55	full flask, sampling might have stopped	300
Sunday	14.4.2002	7h30		n.a
Sunday	21.4.2002	7h45		308
Monday	22.4.2002	8h00	flask broke as it was changed, water was filled in another flask form metallic container	315
Tuesday	23.4.2002	end of sampling 7h30		

n.a: not analysed

**Treated Waste Water (Sampler type: IBUK-KS 350, IBUK PNV-MC)**

Sampling proportional to the flow, every 2 impulsions (1 impulsion =10 m<sup>3</sup>) a sample is taken, Quantity sampled per impulsion: 75 ml

Day	Date	Start time	remarks	sample number
Tuesday	9.4.2002	7h35		271
Wednesday	10.4.2002	8h00		278
Thursday	11.4.2002	7h50		287
Friday	12.4.2002	7h47		294
Saturday	13.4.2002	6h50	full flask, sampling might have stopped	301
Sunday	14.4.2002	7h25		n.a
Sunday	21.4.2002	7h50		309
Monday	22.4.2002	8h05		316
Tuesday	23.4.2002	Fin prélèvement 7h35		

n.a: not analysed

**Primary Sludge**

Day	Date	Sampling time	number of samples taken	volume per sample (3l)	remarks	sample number
Wednesday	10.4.2002	8h48	8	2	mixing 30 min, thickener left	272
Thursday	11.4.2002				thickener middle	279
Friday	12.4.2002	9h00	8	2	mixing 30 min., thickener right	288
Saturday	13.4.2002	8h20	8	2	mixing 30 min, thickener left	295
Sunday	14.4.2002	9h15	8		mixing 25 min, thickener middle	302
Monday	22.4.2002	10h00	8		mixing 25 min, thickener left	310
Tuesday	23.4.2002	9h00	9		mixing 25 min, thickener middle	317

**Supernatants Thickener**

Day	Date	Sampling times	# of samples taken	volume per sample	remarks	sample number
Wednesday	10.4.2002	7h45,8h07	4/1	1 litre	last two samples contained some sludge	273
Thursday	11.4.2002	7h55,8h10,8h13,8h30,	1,2,2,2	1 litre		280
Friday	12.4.2002	7h30,7h45,8h05,8h11	2,2,2,2	1 litre		289
Saturday	13.4.2002	7h05-7h50 about every 5 min.	2,2,2,2,1,1	1 litre		296
Sunday	14.4.2002	7h50-8h55 about every 5 min.	12	1 litre	last sampled contained some sludge	303
Monday	22.4.2002	8h30- 9h30 about every 5 min.	1,1,1,1,1,2,2,2	1 litre		311
Tuesday	23.4.2002	7h45- 8h30 about every 5-10 min	1,1,1,1,2,2,2	1 litre		318

**Activated Sludge**

Day	Date	Sampling times	# of samples taken	volume per sample	remarks	sample number
Tuesday	9.4.2002	16h50	7	1 litre		274
Wednesday	10.4.2002	8h30/9h00/9h10/9h25/16h30	2/2/1/1,7	2 litres		284
Thursday	11.4.2002	8h05,8h20,8h30,8h45, 16h30	1,1,1,1,1,7	2 litres		290
Friday	12.4.2002	7h30,8h00,8h10,8h30,16h30	1,1,1,1,7			297
Saturday	13.4.2002	7h15 –8h15 about every 5 min	1,2,2,2,2,2,1,1,7			304
Sunday	14.4.2002	7h55-8h50	2,1,1,1,1,1,1,1	1 litre		n.a
Sunday	21.4.2002	12h00, 14h00, 16h00, 18h00, 21h00, 23h00, 01h00, 03h00, 8h00	9*2			312
Monday	22.4.2002	19h30, 20h00, 21h00, 22h00, 23h00, 2h00, 8h30, 9h30	2,1,1,1,1,1,1,1			319

### Supernatants from Storage Tank

Day	Date	Sampling times	# of sam- ples taken	volume per sample	remarks	sample number
Wednesday	10.4.2002	8h25/8h32/8h58/9h05/9h20	1/1/2/1/2	1 litre	sampled from both storage tanks	275
Thursday	11.4.2002	8h00,8h15,8h17,8h40,9h00	2,2,2,2,2	1 litre	sampled from both storage tanks	282
Friday	12.4.2002	7h35,7h50,8h05,8h15h8h25	2,2,2,2,1	1 litre	sampled from right storage tank	291
Saturday	13.4.2002	7h10-8h00 about every 5 min.	1,2,2,2,2,1,1	1 litre	sampled from right storage tank	298
Sunday	14.4.2002	-	-	-	no pumping	
Monday	15.4.2002	-	-	-	no pumping	
Tuesday	16.4.2002	7h50-8h40	2,2,2,1,1,1,1	1 litre	sampled from right storage tank	305
Monday	22.4.2002	8h30- 9h30 about every 5 min.		1 litre	sampled from the right storage tank while sludge was transferred from the digester	3113
Tuesday	23.4.2002	7h45 - 8h30 et 9h30 about every 5-10 min.	1,1,1,1,1,1,1,1,1,1	1 litre	sampled from the right storage tank while sludge was transferred from the digester	320

#### Remarks:

PVC tube of the waste water sampler exchanged the 11.4.2002.

The tubes of the other automatic samplers were made from PVC.

There were spring holidays in most of the public schools in the catchment's area in the beginning of the sampling period with the back to school day on the 21st of April.

## Appendix 3.5 Effect of Freezing Water Samples

### Description of the Experiment

A sample of the discrete water sample, taken the last day of the sampling period was frozen for 50 hours (-20°C). Afterwards the sample was defrosted at ambient temperature. In the meantime another sample of the same original sample was stored in the refrigerator at 7°C. Both samples were extracted and analysed. The GC-MS injection sequence was: non frozen to stabilise (twice), standard solution, non frozen to analyse, frozen to analyse and standard solution. Results can be found in Table 3.5.1 Since it was found that the peak areas of the standard solution can vary substantially (Appendix 3.10) between different injections, the variation between the two standard injections is given in the last column of this table, in order to ensure the stability of the GC-MS.

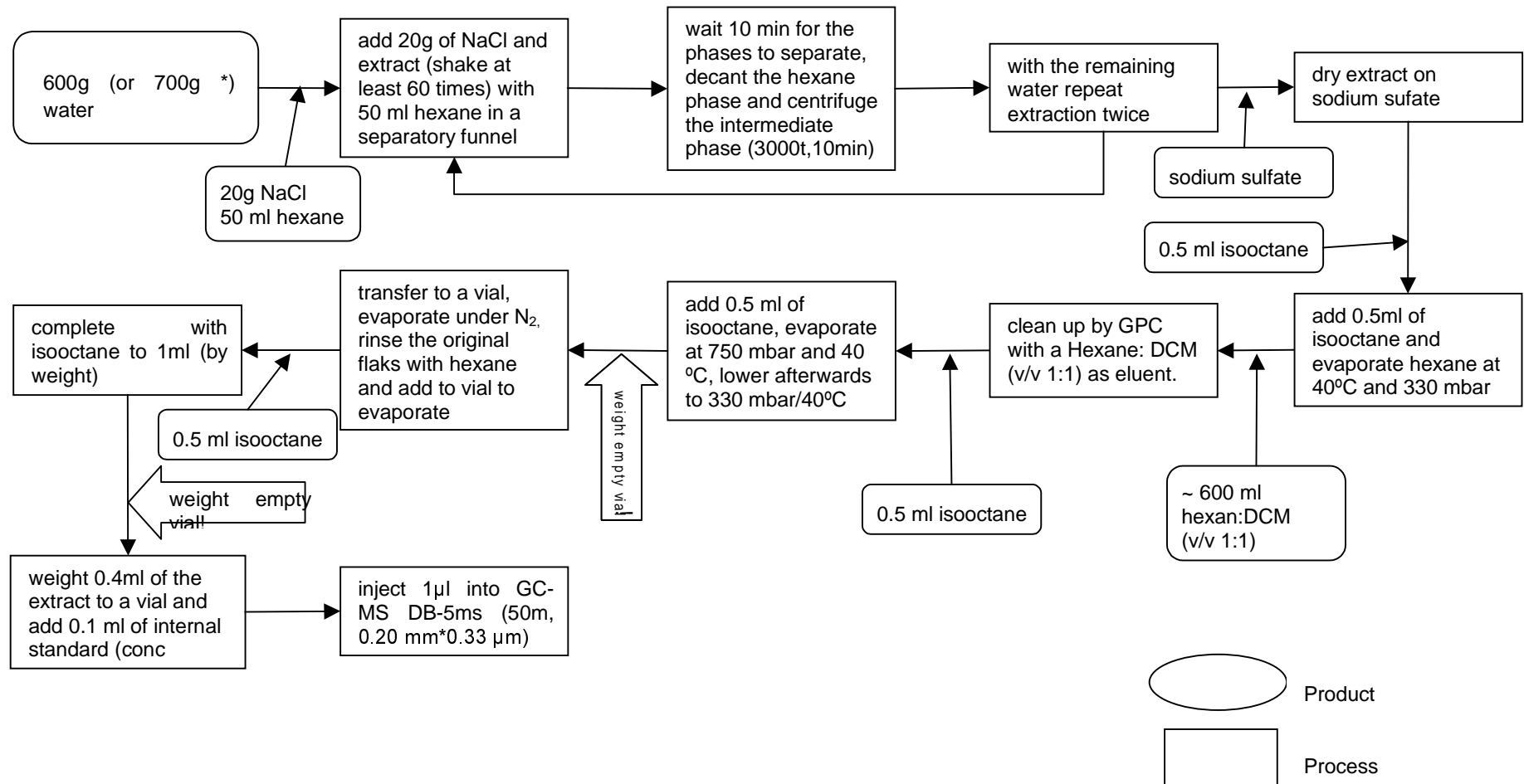
Table 3.5.1 Effect of freezing a waste water sample on the polycyclic musk concentrations

	frozen sample	non frozen sample	frozen/non frozen	variation of the std solution
	conc ng/l	conc ng/l		
Cashmeran	31.60	21.90	-44%	5%
Celestolide	164.36	170.86	4%	1%
Phantolide	69.79	72.42	4%	1%
Traseolide	132.47	142.10	7%	0%
Galaxolide 213	13597.79	13778.12	1%	0%
Tonalide 258	2793.42	3041.85	8%	1%
Galaxolidone	233.20	104.19	-124%	5%

As can be seen from Table 3.5.1 concentrations found in the non frozen sample are only slightly higher than concentrations measured in the frozen sample for all polycyclic musk compounds except Cashmeran and Galaxolidone. Variation is too little in order to claim an effect of freezing a water sample and therefore concentrations found in environmental samples were not corrected by a freezing factor.

The concentrations of Cashmeran and Galaxolidone are higher in the frozen than in the no frozen sample. If compared with other analysis of this specific water sample (Table 3.7.2, Cashmeran concentration of 33.5 and 33 ng/l and Galaxolidone 287 and 299 ng/l) it can be seen, that the values found in the non frozen sample are extraordinary low. Therefore it is assumed that there was a problem with the analysis of these two compounds in the no frozen sample. A possible difficulty might have been the clean-up by GPC, because the two compounds Cashmeran and Galaxolidone are eluted at last and for some reason might have remained in the column (Appendix 3.12). Due to time restrictions it was not possible to repeat the experiment and it is assumed that freezing does not change polycyclic musk concentrations in water for all compounds

### Appendix 3.6 Protocol to Analyse Polycyclic Musks in Water Samples of a Waste Water Treatment Plant







**Glassware to rinse**

- 1 Squibb separatory funnel (1l) with glass stopper
- 1 Beakers (50 ml) to weight salt
- 1 spatula
- 1 Erlenmeyer flask (1l) with glass stopper
- 1 funnel
- 1 centrifugation tube with lid
- 2 round bottom flask (250ml) with glass stopper

**Chemicals**

- standard solution Cashmeran (0.031 $\mu$ g/g), Celestolide (0.145 $\mu$ g/g), Phantolide (0.07 $\mu$ g/g), Traseolide (0.12 $\mu$ g/g), Galaxolide (13.74 $\mu$ g/g), Tonalide (2.33 $\mu$ g/g), Galaxolidone (0.23 $\mu$ g/g) in isooctane
- internal standard solution Tonalide D3 in isooctane, concentration 4 $\mu$ g/g
- 20g NaCl
- 3 spoons Na<sub>2</sub>SO<sub>4</sub>
- 180ml hexane
- 2 ml isooctane
- 650 ml Hexane:DCM (v/v 1:1)

**others**

- glasswool
- several pasteur pipettes
- 2 vials
- GPC (column: 600mm x 20 mm, filled with 100 g of Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA), flow rate 5ml/min)
- rotavap

**Operation mode**

Weight 600ml (or 700 ml\*) in an Erlenmeyer flask. Pour into the separatory funnel and add NaCl. Put 50ml hexane and shake well, while releasing gases by opening the valve from time to time. Shake at least 60 times. Leave it for at least 10 min for the phases to separate. Decant water into the Erlenmeyer flask, drain intermediate, emulsionary phase to the centrifugation tube and release hexane phase over sodium sulphate into the round bottom flask. Pour water back into the separatory funnel, add 50 ml hexane and shake well again. Don't forget to release the gases! Decant the water into the Erlenmeyer, the intermediate phase into the centrifugation tube and release the hexane phase over sodium sulphate into the round bottom flask. Repeat extraction once more. Rinse separatory funnel with about 7ml hexane and add it to the extract in the round bottom flask. Centrifuges intermediate phase at 3000 turns for 10 min. Pipette the hexane phase over sodium sulphate into the round bottom flask. Rinse sodium sulphate with about 3ml of hexane. Add 0.5 ml of isooctane and evaporate at 330mbar and 40 °C to 0.5ml. Condition GPC for 20 min. Fill extract into the injection syringe of the GPC. Rinse round bottom flask twice with 1.5ml of hexane: DCM and add it to the extract in the syringe. Complete the content in the syringe to 5ml. Rinse injector of GPC twice before injection. Inject extract into the GPC and rinse injector after 10min. Dispose

the first 175ml (35min) to the waste and collect the following 225ml (45min) into a round bottom flask. Add 0.5ml of isooctane and evaporate at 750mbar and 40°C. Lower pressure to 330mbar after all DCM has been evaporated. Transfer the extract (about 0.5ml) to a vial (which was weighted before) and rinse the round bottom flask twice with about 1.5ml of hexane. Add the washings to the vial while the extract is evaporated under a gentle stream of nitrogen. Evaporate to less than 0.5ml but not to dryness. Complete to 1ml by weight. Weight another vial and transfer 0.4ml (by weight) of the extract to it. Add 0.1 ml of the internal standard (µg/g) and inject as soon as possible into the GC-MS (analyse mode and temperature programme see [Appendix 2.9](#))

**Calculation:**

$$\frac{\text{result\_of\_GC-MS}(\text{ng/g}) * (\text{endvolume\_in\_vial}(\text{g isooctan})) * (\text{volume\_of\_1ml\_extract}(\text{g isooctane})) * 1000 * 1000}{(\text{weight\_of\_0.4ml\_extract}(\text{g isooctane})) * \text{volume\_sewage}(\text{g})} = \text{concentration\_in\_water}(\text{ng/l})$$

### Appendix 3.7 Variation of Polycyclic Musk Concentrations Found with SPE or GPC as Clean-up Method

Kupper (2002) published that it was possible to inject extracts of waste water as sludge samples into the GC-MS without clean-up. This method was performed in this study but there remained considerable contamination in the column even after injecting standard solutions for several times. In order to improve analysis, it was looked for a clean-up method. As suggested by Berset (2002), the solid phase extraction method was applied. After the column (0.5g SiOH, column purchased from Macherey-Nagel, Düren, Germany) had been conditioned with 10 ml DCM (Romil), the cartridge was loaded with the extract. 10 ml were used to elute the extract. This method had been approved by loading the standard solutions and determining recovery rates. However, it was found that with the SPE clean-up method the results of different injections of the same vial in the GC-MS (Table 3.7.1) and the results of different extractions of the same water sample (Table 3.7.2) vary substantially. According to the results found in Table 3.7.1 it can even be suspected that there is a degradation process taking place in the vial.

Table 3.7.1: Concentrations (ng/l) found by different injections of the same vial, in the GC-MS, cleaned up with SPE.

	one night refrigerated before analysis (SPE)	analysed three days after extraction (SPE)	analysed four days after extraction (SPE)	mean	standard deviation (% of the mean)
	13060202	14060217/18/22	16060214/17		
Cashmeran	51.99	35.84	27.78	38.54	32%
Celestolide	206.98	145.76	110.18	154.31	32%
Phantolide	77.30	51.17	45.36	57.94	29%
Traseolide	182.76	127.58	99.34	136.56	31%
Galaxolide 213	18735.76	10597.27	7469.10	12267.37	47%
Tonalide 258	4120.32	2676.35	1919.26	2905.31	38%
Galaxolidone	1281.04	679.03	369.94	776.67	60%

Table 3.7.2: Concentrations (ng/l) found in different extractions of the same water sample, cleaned up by SPE. Remarkable are the variations for Cashmeran and Galaxolidone. If the highest values are not taken into account, variation decreases to 0% and 3% respectively.

	one night refrigerated before analysis (SPE)	extraction and injection 16.06.02 (SPE)	extraction and injection 20.06.02 (SPE)	mean	standard deviation (% of the mean)
	13060202	16060226	20060204/06/12		
Cashmeran	51.99	33.45	33.37	39.61	27%
Celestolide	206.98	154.90	162.70	174.86	16%
Phantolide	77.30	87.85	95.08	86.74	10%
Traseolide	182.76	135.92	144.60	154.43	16%
Galaxolide 213	18735.76	14587.35	15110.01	16144.37	14%
Tonalide 258	4120.32	2854.29	3086.21	3353.61	20%
Galaxolidone	1281.04	287.25	298.91	622.40	92%

In order to stabilise the results, water extracts were cleaned up with GPC instead of SPE. Gel permeation chromatography system consisted of a Perkin-Elmer, Serie 4 pump with a 5ml sample loop, a column (600mm x 20 mm) that was filled with 100 g of Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) and a Gilson fraction collector, model 202. N-Hexane: Dichloromethane (50:50 v/v) was used as eluent at a flow rate of 5 ml/min. The optimum sampling window was between 45 and 80 min (225 and 400 ml). As can be seen from Table 3.7.3 and Table 3.7.4 the results obtained from two injections of the same vial do vary less than if samples are cleaned up with SPE (Table 3.7.1). Less variation was also found if the same water sample was extracted several times (Table 3.7.5). These results conducted to the choice of GPC clean up method for water as for sludge samples, even it is more time and solvent consuming.

Table 3.7.3: Concentrations (ng/l) found if the same extract (waste water spiked with 50% of its concentration) was injected 10 days later

	spiked water 2, GPC, extraction and injection 2.7.02	spiked water 2, GPC, extraction 2.7.02, injection 12.7.02		mean	standard deviation (% of the mean)
	2070206	12070224			
Cashmeran	57.14	59.90		58.52	3%
Celestolide	277.68	256.47		267.08	6%
Phantolide	114.78	105.69		110.23	6%
Traseolide	221.13	209.67		215.40	4%
Galaxolide 213	22114.14	20723.75		21418.95	5%
Tonalide 258	4710.51	4448.65		4579.58	4%
Galaxolidone	810.61	649.71		730.16	16%

Table 3.7.4: Concentrations (ng/l) found if the same extract (waste water spiked with 100% of its concentration) was injected 10 days later

	spiked water 3, GPC, extraction and injection 2.7.02	spiked water 3, GPC, extraction 2.7.02, injection 12.7.02		mean	standard deviation (% of the mean)
	2070207	12070225			
Cashmeran	82.13	82.69		82.41	0%
Celestolide	346.63	323.82		335.22	5%
Phantolide	140.44	136.02		138.23	2%
Traseolide	278.44	272.64		275.54	1%
Galaxolide 213	27954.41	27222.09		27588.25	2%
Tonalide 258	5871.32	5806.79		5839.05	1%
Galaxolidone	1293.23	1091.56		1192.39	12%

Table 3.7.5: Concentrations (ng/l) found in different extractions of the same water sample, cleaned up by GPC. The variations for Cashmeran and Galaxolidone are quite high but from the three extractions the second found unusual low values for these compounds. If these two values are not taken into account, variation decreases to 1% and 2% respectively.

	extraction 20.06.02 injection 26.06.2002 (SPE/GPC)	extraction and injection 27.06.02 (GPC)	extraction and injection 2.7.02 (GPC)	mean	standard deviation (% of the mean)
no. Analysis	26060204	26060218/20	2070205		
Cashmeran	31.13	21.90	31.39	28.14	19%
Celestolide	145.03	170.86	166.53	160.81	9%
Phantolide	87.69	72.42	75.16	78.42	10%
Traseolide	132.25	142.10	135.36	136.57	4%
Galaxolide 213	13119.55	13778.12	14403.64	13767.10	5%
Tonalide 258	2763.48	3041.85	2842.05	2882.46	5%
Galaxolidone	230.58	104.19	223.30	186.02	38%

### Appendix 3.8 Effect of Total Evaporation under Nitrogen on the Polycyclic Musk Concentration

Berset (2000) reported that careful evaporation under N<sub>2</sub> was crucial. In order to assess the effect of evaporation to dryness, the following experiment was carried out. 0.5 ml of a mixture of all standard solutions was pipetted into 100 ml hexane:DCM (v/v 1:1) and 0.5 ml of isooctane was added. Evaporation was conducted under 750 mbar and at 40°C. The pressure was lowered to 550 mbar and 330mbar in order to evaporate hexane. The remaining 0.5 ml was transferred to a vial and the round bottom flask was rinsed with hexane that was added to the vial to evaporate under nitrogen. One of the two samples was evaporated to dryness whereas the other sample was not. The recovery rates were determined in comparison to the original concentration found in the standard mixture and can be found in Table 3.8.1.

Table 3.8.1: Recovery rates of standard solutions if evaporated to dryness or not

	evaporated to dryness under nitrogen	evaporated <b>not</b> to dryness under nitrogen
	29050211	29050212
Cashmeran	5%	85%
Celestolide	36%	86%
Phantolide	42%	86%
Traseolide	54%	87%
Tonalide 258	48%	89%
Galaxoide 213	54%	87%
Galaxolidone	69%	71%

As can be seen from Table 3.8.1 the losses are quite high if the extract is evaporated to dryness above all for Cashmeran. Therefore one needs to watch out during evaporation.

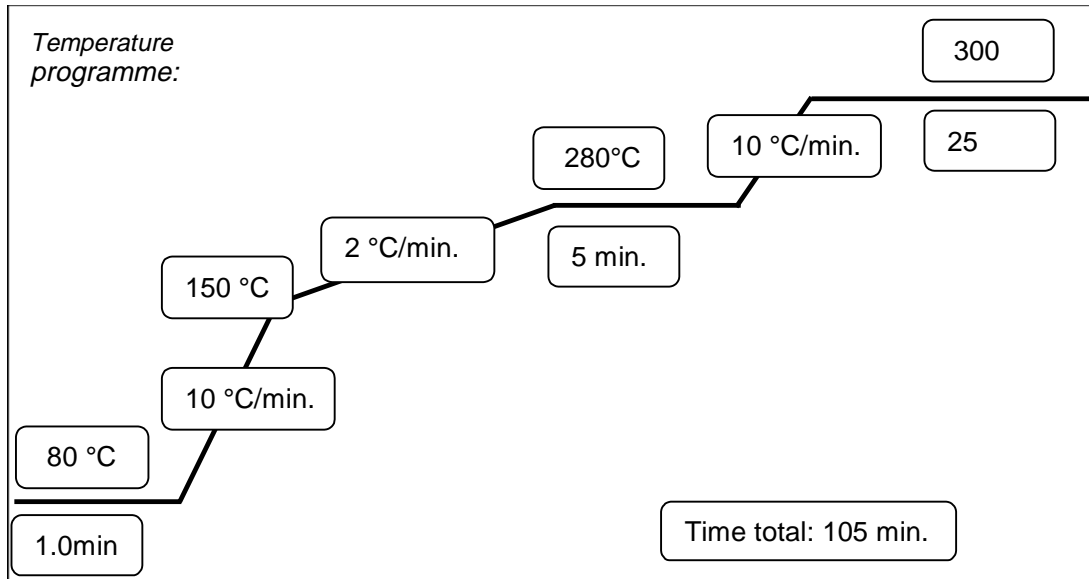
## Appendix 3.9: GC-MS- Programme Standard Solutions

**APPARAT:** HP 5890 - 5971A MSD

**Date:** 14.05.02

**Type of COLUMN:** DB-5ms (50m x 0,20mm x 0,33µm)

no. of serie/article: 9987123



Gas:	He
Head pressure (psi) :	30
Flow (ml/min.) :	0.85 à 80°C
Linear velocity (cm/sec):	26.2 à 80°C

**INJECTOR:**

Type : Splitless



Purge, split (ml/min.) :	39	Split ratio: 45:1	precolumn 0.32mm,
Septum flow (ml/min.) :	5		5m
Volume injected (µl) :	1	A/S injection 10ul	
Type liner :	5181-3316 (with glass wool)		

**DETECTOR:**

Type : MS

Temperature (°C) :	170	transfer	line:
N2, Make-up (ml/min.) :	-	280°C	
N2, Purge anode(ml/min.):	-		
Air 1 (ml/min.) :	-		
Air 2 (ml/min.) :	-		
H2 (ml/min.) :	-		
Air 1 / H2 split	-		
Signal :	-		

**Miscellaneous:**

METHODE MUSCSIM3

**SIM-Ions**

				retention time (min)	starting time (min)
Cashmeran	163.1	191.05	206.15	25.02	20
Celestolide	173.05	229.15	244.15	34.53	30
Phantolide	187.05	229.1	244.15	36.62	35.5
Traseolide	173.15	215.1	258.1	41.08	40
Galaxolide/Tonalide/Tonalide D3	213.15	243.1	258.2	41.55	41.35
Tonalide		243.1	258.2	41.82	41.35
Tonalide D3		246.15	261.15	41.7	41.35
Galaxolidone	257.1	258.1	272	57.55	44

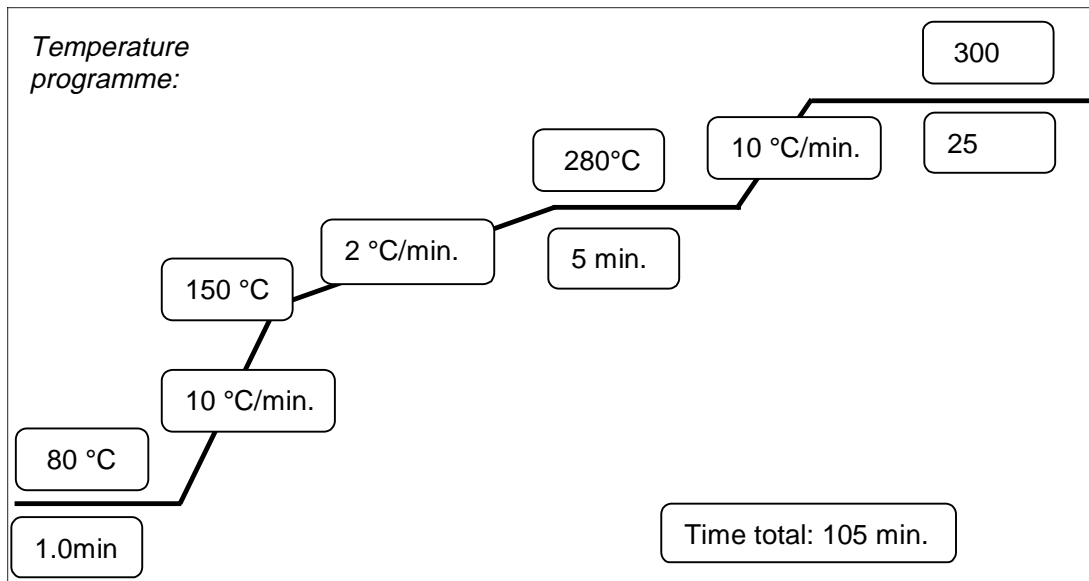
## Appendix 3.9: GC-MS Programme Environmental Samples

**APPARAT:** HP 5890 - 5971A MSD

**Date:** 14.06.02

**Type of COLUMN:** DB-5ms (50m x 0,20mm x 0,33µm)

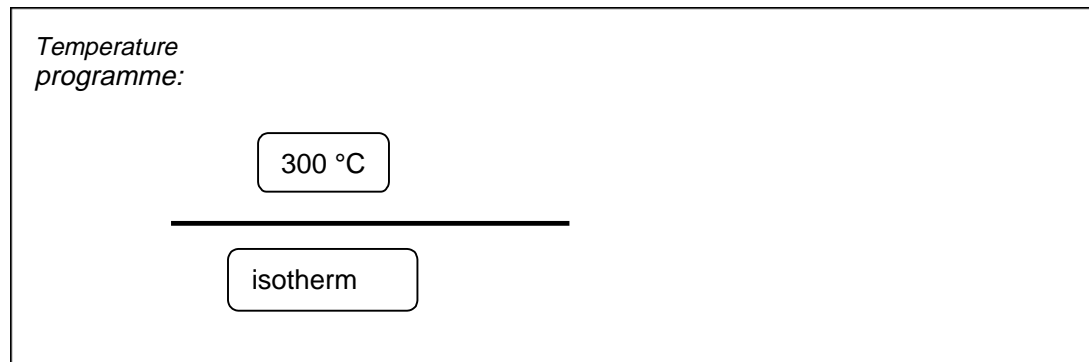
no.of serie/article: 9987123



Gas:	He
Head pressure (psi) :	30
Flow (ml/min.) :	0.85 à
	80 °C
	26.2 à
	80 °C
Linear velocity (cm/sec):	80 °C

**INJECTOR:**

Type : Splitless



Purge, split (ml/min.) :	39	Split ratio: 45:1	precolumn 0.32mm, 5m
Septum flow (ml/min.) :	5		
Volume injected (µl) :	1	A/S injection 10ul	
Type liner :	5181-3316 (with glass wool)		



**DETECTOR:**

Type : MS

Temperature (°C) :	170	transfer	line:
N <sub>2</sub> , Make-up (ml/min.) :	-	280°C	
N <sub>2</sub> , Purge anode(ml/min.):	-		
Air 1 (ml/min.) :	-		
Air 2 (ml/min.) :	-		
H <sub>2</sub> (ml/min.) :	-		
Air 1 / H <sub>2</sub> split	-		
Signal :	-		

**Miscellaneous:**

METHODE MUSCSIME

**SIM-Ions**

				retention time (min)	starting time (min)
Cashmeran	163.1	191.05	206.15	25.02	20
Celestolide	173.05	229.15	244.15	34.53	30
Phantolide	187.05	229.1	244.15	36.62	35.5
Traseolide	173.15	215.1	258.1	41.08	40
Galaxolide/Tonalide/Tonalide D3	213.15	243.1	258.2	41.55	41.35
Tonalide		243.1	258.2	41.82	41.35
Tonalide D3		246.15	261.15	41.7	41.35
Galaxolidone	257.1	258.1	272	57.55	44

## Appendix 3.10 Stabilisation of the GC-MS with Environmental Samples

The stability of the GC-MS was tested by injecting twice a standard solution (mélange 2/10), then a waste water extract (injection 29050207, which is not represented in Figure 3.10.1) and afterwards again twice the same standard solution. As can be seen in Table 3.10.1 and Figure 3.10.1, the peak areas of the standard solution vary greatly. Therefore it was tried to stabilise the GC-MS by the injection of an environmental sample before injection the standard solution as a reference. As can be seen from Figure 3.10.2 and Table 3.10.1 the peak areas of the standard solution vary much less than without stabilisation.

Table 3.10.1: Concentration of the different polycyclic musk compounds in the standard solution and maximal variation of the peak areas in the GC-MS output if starting with the standard solution or if stabilising with an environmental sample

	mél 2/10 conc (ng/g)	max. difference of the peak areas without stabilisation	max. difference of the peak areas with stabilisation
Cashmeran	75.03	18%	9%
Celestolide	75.70	30%	17%
Phantolide	52.08	24%	16%
Traseolide	84.71	33%	23%
Galaxolide 213	78.42	22%	11%
Tonalide 258	94.45	29%	20%
Galaxolidone	123.39	90%	91%

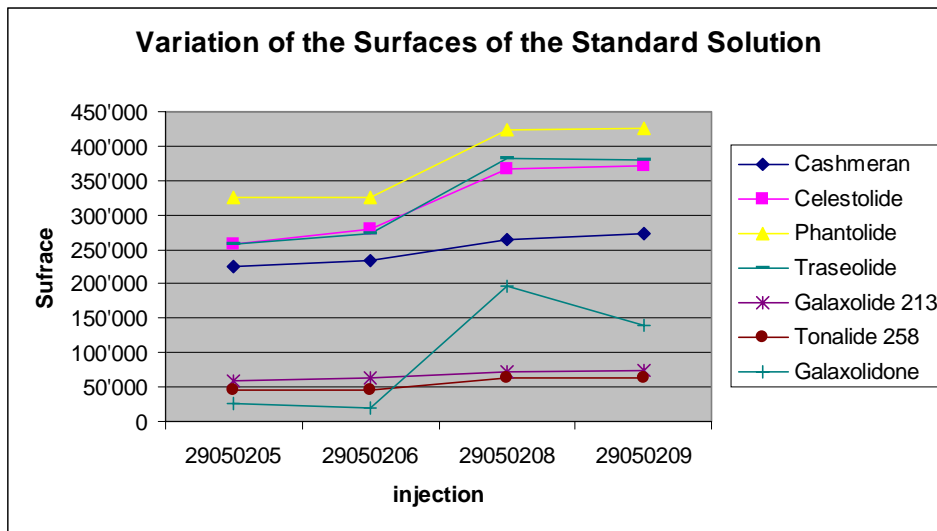


Figure 3.10.1

Variation of the peak areas in the GC-MS output if starting with the standard solution

The results represented in Figure 3.10.1 were obtained with the following sequence:

29050205	standard solution mélange 2/10
29050206	standard solution mélange 2/10
29050207	waste water extract
29050208	standard solution mélange 2/10
29050209	standard solution mélange 2/10

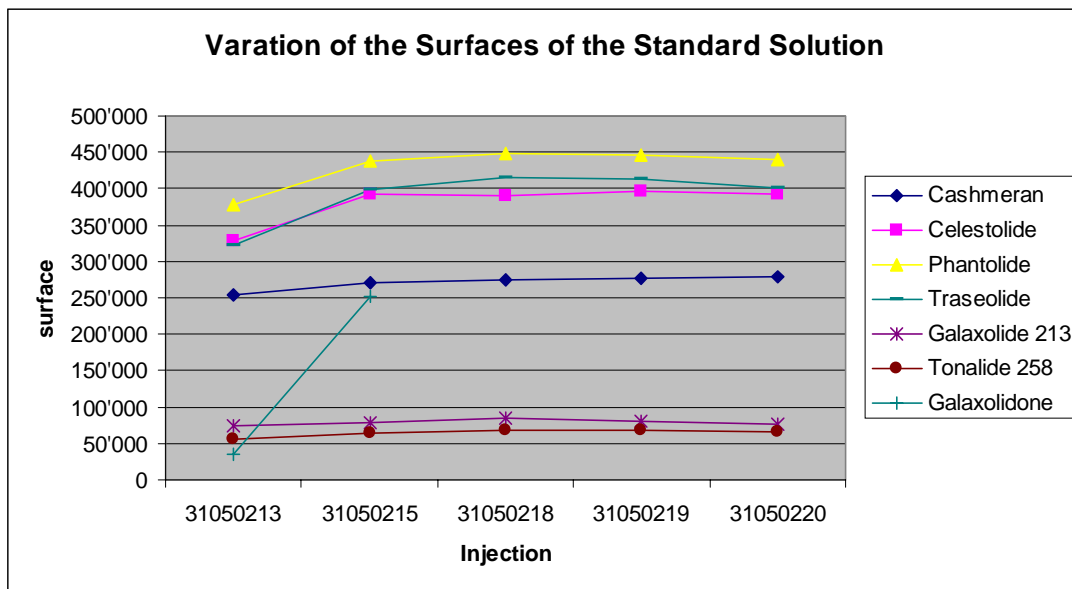


Figure 3.10.2

Variation of the peak areas in the GC-MS output if starting with a water extract  
The results outlined in Figure 3.10.2 were obtained with the following sequence

31050213	standard solution mélange 2/10
31050214	waste water extract
31050215	standard solution mélange 2/10
31050216	waste water extract
31050217	waste water extract
31050218	standard solution mélange 2/10
31050219	standard solution mélange 2/10
310502120	standard solution mélange 2/10

As can be seen in Figure 3.10.2 the peak areas of the standard solution rise after the first injection of the waste water extract (injection 31050215). After that the peak areas remain stable, even though the water extract was injected twice before the standard solution was reinjected, indicating a stable analysis. Unfortunately there was interference in injection 31050218 at the elution time of Galaxolidone. Therefore stability for this compound could not be assessed in this experiment.

For the analysis of environmental samples the GC-MS was always stabilised twice by the injection of the supposedly most contaminated water extract. Afterwards the standard solution was injected once. This injection was followed by two environmental samples and another injection of the standard solution. If more than two environmental samples was analysed in a sequence, two more extract were injected before another analysis of the standard solution. This sequence allowed to ensure low variation of the standard solution (> 5%), hence a stable analysis of the environmental samples.

If the peak areas of the standard solution mélange 2/10 of the sequence with and without stabilisation by an environmental sample are compared, it can be seen that they increased considerably. However this does not imply that the detection limit increased as well, because noise could have increased also.

### Appendix 3.11 Effect of the Temperature of the Injector on the Identification of Polycyclic Musks

Even the concentration of Galaxolidone was about 25 times higher than the concentrations of the other compounds in standard mixture mélange 1, the peak area representing Galaxolidone was quite low. In the beginning it was worked with a normal injector temperature of 250°C. In communication with Dr. Jean-Daniel Berset, it was found that a change of the temperature of the detector could improve the situation. The temperature was lowered and increased. As can be seen in Table 3.11.1 the sensitivity for Galaxolidone rose considerably if the temperature of the injector was increased. The peak areas of the other compounds did not change at higher injector temperature. Due to the materials used in GC-MS temperature could not been risen higher than 300°C and therefore this temperature was chosen for the further analysis. Unfortunately not all peak areas are available for an injection temperature of 200°C because not all SIM ions were entered correctly in this programme.

Table 3.11.1: Effect of the temperature of the injector on the detection of polycyclic musks in the GC-MS

		Injector 200°C	Injector 250°C	Injector 280°C	Injector 300°C			
	conc (µg/g)	mélange 1+AHTN D3	mélange 1+AHTN D3	mélange 1+AHTN D3	mélange 1+AHTN D3	difference 200°C/250°C	difference 250°C/280°C	difference 280°C/300°C
		MUSCSIM1	MUSCSIM	MUSCSIM2	MUSCSIM3			
		14050215	15050201	16050201	16050202			
Cashemeran	0.57	wrong ions	1'233'920	1'497'498	1'470'375		121%	98%
Celestolide	0.58	186'919	1'565'347	1'903'263	1'881'702	12%	122%	99%
Phantolide	0.69	194'662	1'835'284	2'267'225	2'232'330	11%	124%	98%
Traseolide	0.88	122'946	2'004'653	2'709'858	2'684'999	6%	135%	99%
Galaxolide 213	0.60	wrong ions	340'376	411'359	404'924		121%	98%
Tonalide D3 261	0.84	wrong ions	333'588	452'059	441'007		136%	98%
Tonalide 258	0.73	15'313	264'421	358'468	359'383	6%	136%	100%
Galaxolidone	19.44	1'192'017	1'817'604	14'044'936	32'747'913	66%	773%	233%

### Appendix 3.12 Determination of the Optimal Sampling Window in the GPC

In order to clean the water extract, they were run through a Gel permeation chromatography system consisting of a Perkin-Elmer, Serie 4 pump with a 5ml sample loop, a column (600mm x 20 mm) that was filled with 100 g of Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) and a Gilson fraction collector, model 202. In order to determine the optimal sampling window 1ml of a mixture of the standard solutions was filled into the injection syringe. The injection volume was completed to 5 ml with hexane: DCM (50:50 v/v). 35 min after injection the first fraction was collected. Afterwards every ten minutes the round bottom flask was exchanged. The fractions were evaporated on the rotavap (770 mbar, 40°C, pressure lowered afterwards to 330 mbar) and under a gentle stream of nitrogen. The peak areas of the different fraction found in the GC-MS are summarised in Table 3.12.1. From these results it is concluded that the optimal sampling window of the GPC lays between 45 and 80 min.

Table 3.12.1: Peak areas of different GPC fractions

	mélange 1 1st injection	mélange 1 2nd injection	mean	F1 (0-10') peak area	F2 (10-20') peak area	F3 (20-30') peak area	F4 (30-45') peak area	recovery rate
content of vial (g)	0.696	0.696		0.691	0.691	0.708	0.703	
	15050209	15050214		15050210	15050211	15050212	15050213	
Cashmeran	1'579'642	1'592'974	1'586'308	0	17'151	1'348'786	27'080	89%
Celestolide	2'137'181	2'145'522	2'141'352	0	547'846	1'390'662	0	91%
Phantolide	2'423'595	2'421'005	2'422'300	0	223'057	1'971'277	0	92%
Traseolide	2'698'438	2'720'842	2'709'640	0	479'449	1'965'517	0	91%
Galaxolide 213	464'487	468'101	466'294	0	19'708	409'633	0	94%
Tonalide 258	326'178	327'806	326'992	0	43'300	252'531	0	92%
Galaxolidone	2'215'994	2'232'567	2'224'281	0	28'916	1'996'207	30'575	94%

### Appendix 3.13 Spiking Experiment for the Entire Analytical Method

In order to assess recovery rates of the analytical method, a spiking experiment was conducted. A discrete waste water sample, taken the last day of the sampling period, was spiked with 50, 100, 150, and 200% of its content of polycyclic musks. The samples were kept in the refrigerator for one hour before they were extracted. As an unspiked sample was analysed as well, expected concentrations in the spiked samples could be calculated by adding the amount of standard added to the amount of polycyclic musks that was already in the waste water sample. Expected and actually measured concentrations are reported in a graph (Figure 3.13.1-Figure 3.13.7) resulting in five points (waste water, spiked 50, 100, 150 and 200%). Recovery rates are determined by drawing a straight line through the five points and by specifying its slope. As can be seen in Table 3.13.1 and Figure 3.13.1-Figure 3.13.7 the deviation of the recovery rates or slopes are low, meaning that recovery rates do not vary a lot.

The concentrations found in the environmental samples analysed were corrected by the recovery rates, meaning that they were multiplied by 1-recovery rate. It is evident that it would have been better to determine recovery rates more than once, but due to time restriction, it was only done once.

Table 3.13.1: Recovery rates, standard deviations thereof and random deviations of the analysis method for polycyclic musks

	recovery rate	standard deviation of recovery rate	random deviation of the straight line (ng/l)
Cashmeran	97.3%	1.61%	1.39
Celestolide	99.9%	4.89%	15.81
Phantolide	92.9%	4.12%	4.74
Traseolide	91.2%	4.69%	13.33
Galaxolide 213	86.3%	3.34%	910.33
Tonalide 258	86.7%	4.26%	256.63
Galaxolidone	92.9%	2.07%	39.16

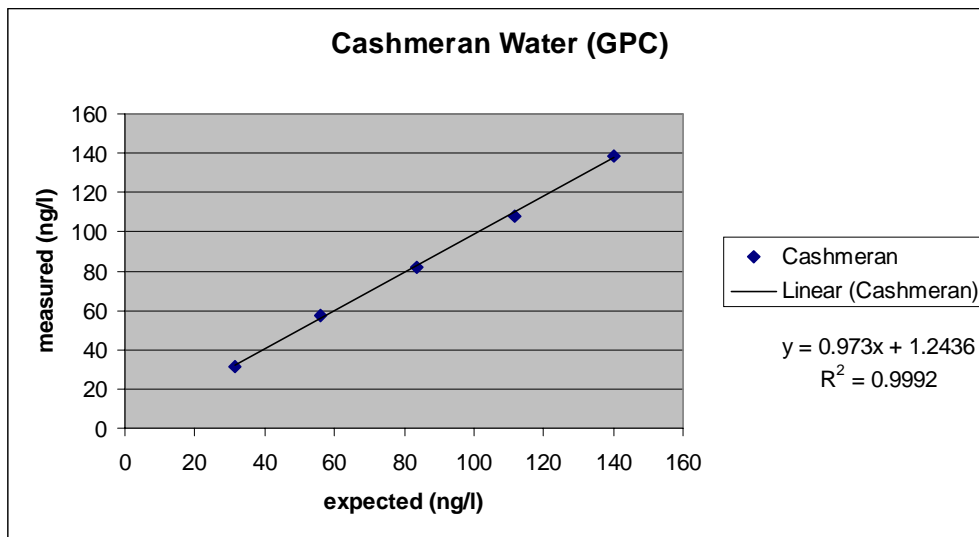
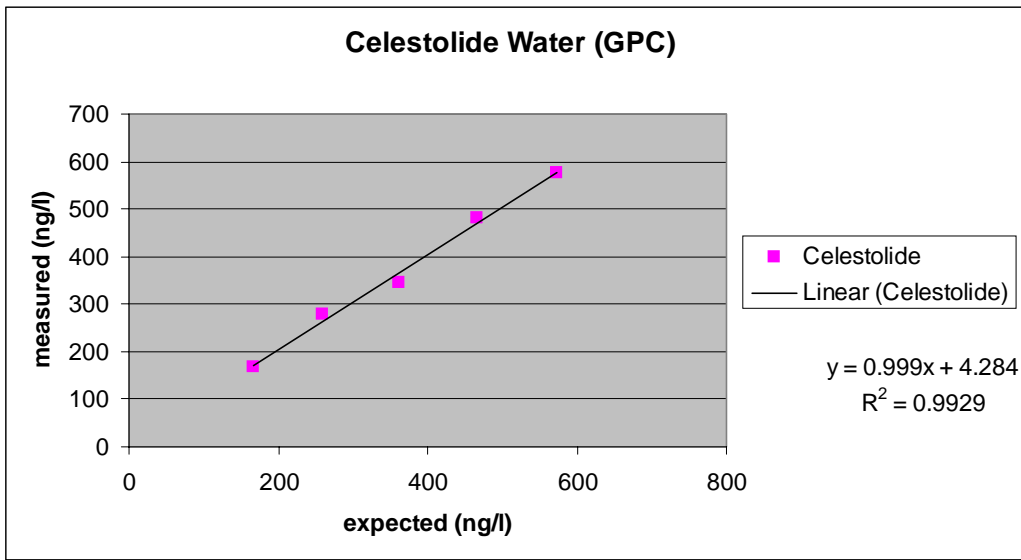


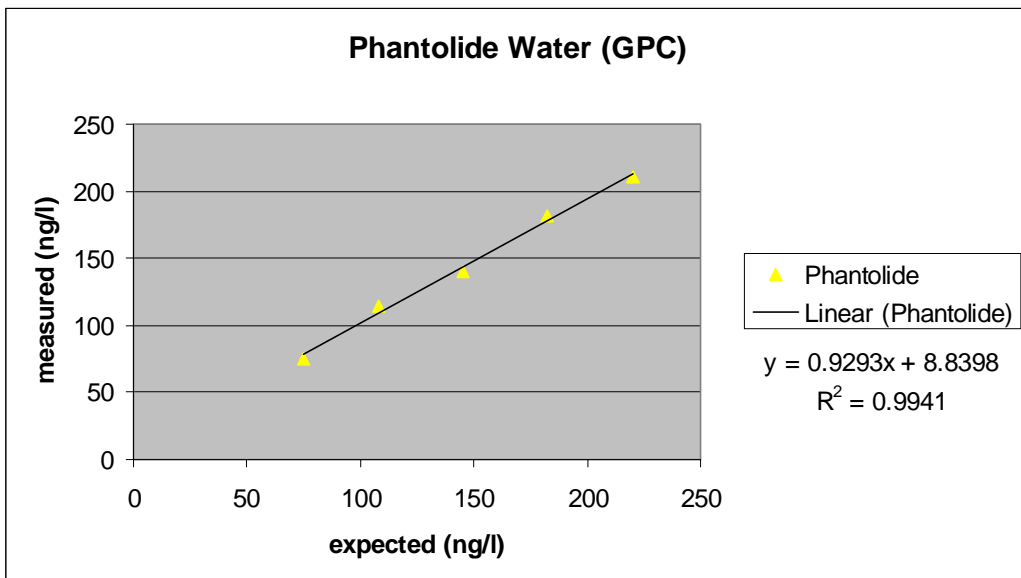
Figure 3.13.1

Recovery experiment in water for Cashmeran (DPMI)



Recovery experiment in water for Celestolide (ADBI)

Figure 3.13.2



Recovery experiment in water for Phantolide (AHMI)

Figure 3.13.3

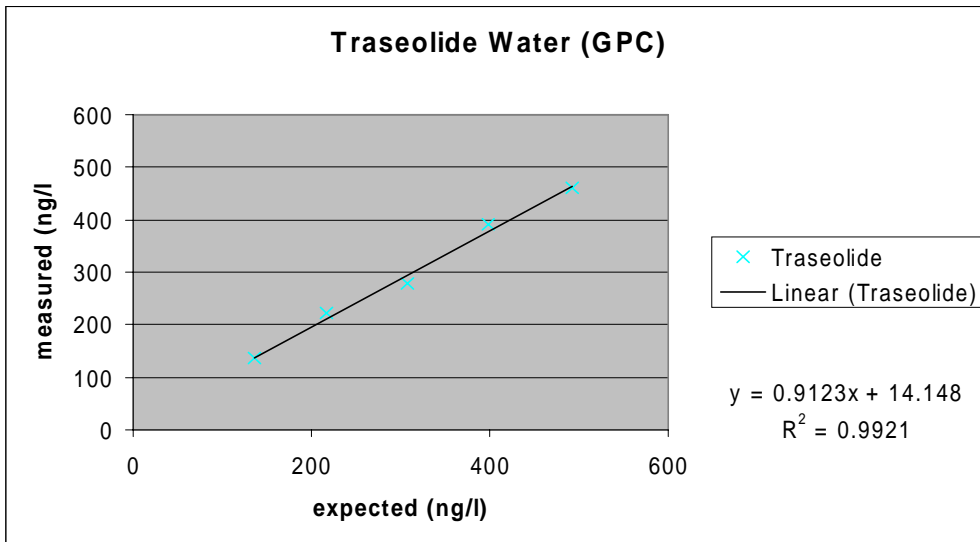


Figure 3.13.4

Recovery experiment in water for Traseolide (AITI)

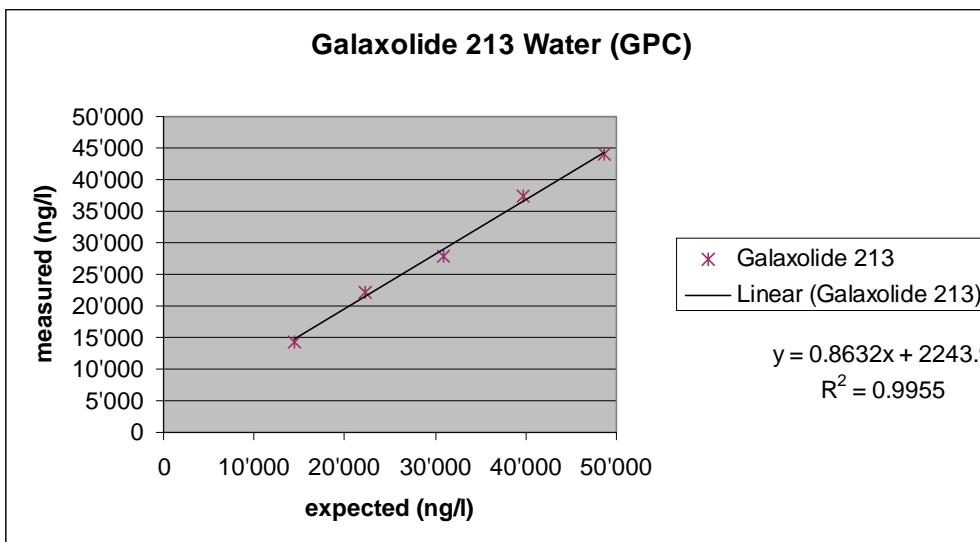


Figure 3.13.5

Recovery experiment in water for Galaxolide (HHBC), extracted ion in GC-MS 213



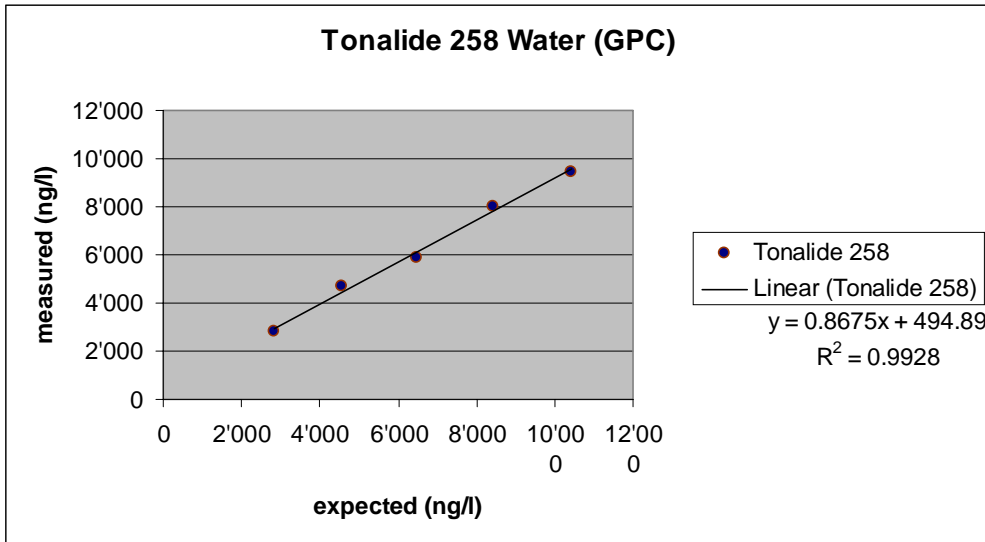


Figure 3.13.6

Recovery experiment in water for Tonalide (AHTN), extracted ion in GC-MS 258

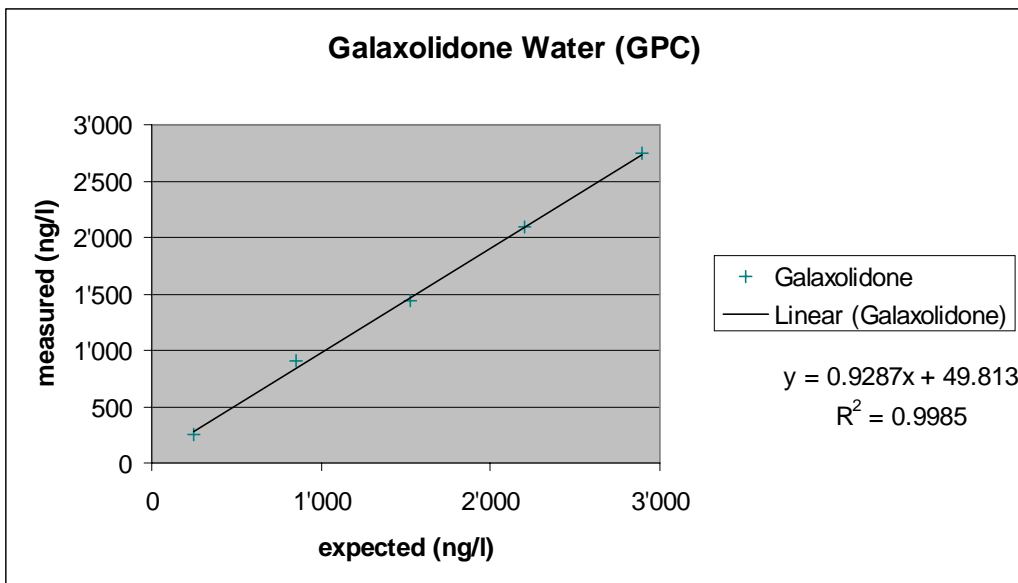


Figure 3.13.7

Recovery experiment in water for Galaxolidone

## Appendix 4.1 Polycyclic Musks Concentrations Measured in Waste Water, Water after Primary Settling, Treated Waste Water, Supernatants from the Thickener and Supernatants from the Sludge Storage Tank

### Waste Water (ng/l)

Table 4.1.1: Concentrations of polycyclic musks found in waste water (ng/l)

sample-Nr.	269	276	285	292	299	307	314				
Date	09.04.2002	10.04.2002	11.04.2002	12.04.2002	13.04.2002	21.04.2002	22.04.2002				
Day	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	mean	standard deviation	median	
Cashmeran	24	29	32	19	27	13	37	26	8	31%	27
Celestolide	196	329	224	140	115	91	154	179	80	45%	154
Phantolide	55	51	105	112	52	31	65	67	30	45%	55
Traseolide	68	71	66	56	67	65	104	71	15	21%	67
Galaxolide	4663	4532	5011	3534	4531	2338	6937	4507	1404	31%	4532
Tonalide	1420	1399	1550	1150	1394	1223	2040	1454	290	20%	1399
Galaxolidone	43	37	36	30	41	0	69	37	20	56%	37

### Water after Primary Treatment (ng/l)

Table 4.1.2: Concentrations of polycyclic musks found in water after primary settling (ng/l)

sample-Nr.	270	277	286	293	300	308	315				
Date	09.04.2002	10.04.2002	11.04.2002	12.04.2002	13.04.2002	21.04.2002	22.04.2002				
Day	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	mean	standard deviation	median	
Cashmeran	19	32	24	33	22	27	31	27	5	19%	27
Celestolide	91	249	133	152	74	66	98	123	63	51%	98
Phantolide	32	49	70	113	39	30	46	54	29	54%	46
Traseolide	43	67	54	68	38	52	69	56	13	23%	54
Galaxolide	2434	3829	3803	3827	2694	2971	4977	3505	871	25%	3803
Tonalide	841	1289	1167	1292	873	978	1486	1132	242	21%	1167
Galaxolidone	31	47	45	47	35	36	63	43	11	25%	45

### Treated Waste Water (ng/l)

Table 4.1.3: Concentrations of polycyclic musks found in treated waste water (ng/l)

sample-Nr.	271	278	287	294	301	309	316				
	09.04.2002	10.04.2002	11.04.2002	12.04.2002	13.04.2002	21.04.2002	22.04.2002	mean	standard deviation	median	
Date	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday				
Day											
Cashmeran	25	24	14	17	17	13	17	18	5	25%	17
Celestolide	36	27	26	34	43	21	25	30	8	25%	27
Phantolide	19	17	14	19	22	12	15	17	3	21%	17
Traseolide	15	10	9	10	11	9	9	10	2	20%	10
Galaxolide	1052	737	582	825	983	619	662	780	182	23%	737
Tonalide	338	234	193	262	305	452	513	328	117	36%	305
Galaxolidone	48	34	30	40	48	32	39	39	7	19%	39

### Supernatants from the Thickener (Thues-Thurs, Fri-Sat, Sun, Mon) (ng/l)

Table 4.1.4: Concentrations of polycyclic musks found in supernatants from the thickener (ng/l)

sample-Nr.	273/280/289	296/303	311	318				
	10.-	13.-	22.04.2002	23.04.2002	mean	standard deviation	median	
Date	12.4.2002	14.4.2002	Sun	Mon				
Day	Tues-Thurs	Fri-Sat						
Cashmeran	50	30	41	56	44	11	25%	45
Celestolide	539	359	237	223	339	147	43%	298
Phantolide	188	196	105	117	152	47	31%	153
Traseolide	253	125	141	158	169	58	34%	149
Galaxolide	14603	8443	9550	9520	10529	2764	26%	9535
Tonalide	5047	2764	3127	3075	3503	1042	30%	3101
Galaxolidone	148	86	140	89	116	33	29%	114

### Supernatants from the Sludge Storage Tank (Wed-Fri, Sat and Tues, Tues, Mon) (ng/l)


Table 4.1.5: Concentrations of polycyclic musks found in supernatants for the storage tank (ng/l)


sample-Nr.	275/282/291	298/320	305	313				
	10.4- 12.4.2002	13.4/23.4.2002	16.04.2002	22.04.2002	mean	standarddeviation		median
Date Day	Wed-Fri	Sat, Tues	Tues	Mon				
Cashmeran	62	58	56	40	54	10	18%	57
Celestolide	139	197	194	148	169	30	18%	171
Phantolide	96	98	104	69	92	15	17%	97
Traseolide	97	123	125	89	108	18	17%	110
Galaxolide	6510	6670	7422	4895	6374	1063	17%	6590
Tonalide	2059	2358	2585	1745	2187	365	17%	2208
Galaxolidone	111	101	121	95	107	11	11%	106

## Appendix 4.2 Results of the one-sided Student T-Test on the Concentrations of Different Types of Water

### Waste Water- Water after Primary Settling


	$\sigma$	$\sigma^*t$ (95%, 12)		diff of means
Cashmeran	3.62	6.45	>	-1.13
Celestolide	38.71	68.98	>	55.44
Phantolide	15.76	28.08	>	12.94
Traseolide	7.46	13.30	<	15.29
Galaxolide	624.62	1'113.08	>	1001.66
Tonalide	142.91	254.67	<	321.28
Galaxolidone	8.69	15.49	>	-6.75


 primary settling has no effect on the musk concentration (95% level)

 primary settling has an effect on the musk concentration (95% level)

### Water After Primary Settling – Treated Waste Water


	$\sigma$	$\sigma^*t$ (95%, 12)		diff of means
Cashmeran	2.60	4.64	<	8.50
Celestolide	24.16	43.05	<	92.96
Phantolide	11.04	19.68	<	37.31
Traseolide	4.85	8.65	<	45.10
Galaxolide	336.30	599.29	<	2725.03
Tonalide	101.65	181.14	<	804.33
Galaxolidone	4.91	8.75	>	4.50


 primary settling has no effect on the musk concentration (95% level)

 primary settling has an effect on the musk concentration (95% level)

### Waste Water - Treated Waste Water

	$\sigma$	$\sigma^*t$ (95%, 12)		diff of means
Cashmeran	3.52	6.27	<	7.37
Celestolide	30.52	54.39	<	148.40
Phantolide	11.39	20.30	<	50.25
Traseolide	5.78	10.30	<	60.39
Galaxolide	535.26	953.83	<	3726.69
Tonalide	118.31	210.84	<	1125.61
Galaxolidone	8.16	14.53	>	-2.25

 primary settling has no effect on the musk concentration (95% level)

 primary settling has an effect on the musk concentration (95% level)

## Appendix 4.3 Transfer Coefficients of Polycyclic Musks during the Waste Water Treatment Process

### Waste Water -> Water after Primary Settling

Table 4.3.1: Daily transfer coefficients between waste water and water after primary settling

	269/270 10.04.2002 Tuesday	276/277 11.04.2002 Wednesday	285/286 12.04.2002 Thursday	292/293 13.04.2002 Friday	299/300 14.04.2002 Saturday	307/308 22.04.2002 Sunday	314/315 23.04.2002 Monday	TC of mean	mean TC	standard deviation
Cashmeran	19%	-11%	24%	-76%	17%	-109%	17%	-4%	-17%	54%
Celestolide	54%	24%	40%	-8%	36%	28%	36%	31%	30%	19%
Phantolide	42%	4%	33%	-1%	24%	2%	29%	19%	19%	17%
Traseolide	37%	5%	19%	-22%	44%	21%	34%	22%	20%	23%
Galaxolide	48%	16%	24%	-8%	41%	-27%	28%	22%	17%	27%
Tonalide	41%	8%	25%	-12%	37%	20%	27%	22%	21%	18%
Galaxolidone	29%	-25%	-22%	-57%	13%	#DIV/0!	8%	-18%	-9%	31%

### Waste Water -> Treated Waste Water

Table 4.3.2: Daily transfer coefficients between waste water and treated waste water

	269/271 10.04.2002 Tuesday	276/278 11.04.2002 Wednesday	285/287 12.04.2002 Thursday	292/294 13.04.2002 Friday	299/301 14.04.2002 Saturday	307/309 22.04.2002 Sunday	314/316 23.04.2002 Monday	TC of mean	mean TC	standard deviation
Cashmeran	-5%	17%	56%	7%	36%	-5%	54%	29%	23%	26%
Celestolide	82%	92%	88%	76%	63%	77%	84%	83%	80%	10%
Phantolide	65%	68%	86%	83%	57%	61%	78%	75%	71%	11%
Traseolide	78%	86%	86%	82%	84%	86%	91%	85%	85%	4%
Galaxolide	77%	84%	88%	77%	78%	74%	90%	83%	81%	6%
Tonalide	76%	83%	88%	77%	78%	63%	75%	77%	77%	8%
Galaxolidone	-13%	9%	17%	-35%	-18%	#DIV/0!	43%	-6%	1%	28%

### Water after Primary Settling -> Treated Waste Water

Table 4.3.3: Daily transfer coefficient between waster after primary settling and treated waste water

	270/271 10.04.2002 Tuesday	277/278 11.04.2002 Wednesday	286/287 12.04.2002 Thursday	293/294 13.04.2002 Friday	300/301 14.04.2002 Saturday	308/309 22.04.2002 Sunday	315/216 23.04.2002 Monday	TC of mean	mean TC	standard deviation
Cashmeran	-30%	25%	42%	47%	23%	50%	44%	32%	29%	28%
Celestolide	60%	89%	81%	77%	42%	68%	75%	75%	70%	15%
Phantolide	40%	66%	79%	84%	43%	60%	68%	69%	63%	17%
Traseolide	65%	85%	83%	85%	72%	82%	87%	81%	80%	8%
Galaxolide	57%	81%	85%	78%	64%	79%	87%	78%	76%	11%
Tonalide	60%	82%	84%	80%	65%	54%	65%	71%	70%	12%
Galaxolidone	-58%	27%	32%	14%	-36%	12%	38%	10%	4%	37%

## Appendix 4.4: Coefficient of Determination between Polycyclic Musk Concentrations in Waste Water, Waste Water after Primary Settling and Treated Waste Water

### Waste Water

Table 4.4.1: Coefficient of determination between polycyclic musk concentrations in waste water

	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Galaxolidone
Cashmeran	1						
Celestolide	0.21	1					
Phantolide	0.05	0.01	1				
Traseolide	0.47	0.00	0.05	1			
Galaxolide	0.90	0.08	0.03	0.66	1		
Tonalide	0.72	0.01	0.00	0.89	0.85	1	
Galaxolidone	0.79	0.07	0.05	0.52	0.93	0.67	1

### Water after Primary Settling

Table 4.4.2: Coefficient of determination between polycyclic musk concentrations in water after primary settling

	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Galaxolidone
Cashmeran	1						
Celestolide	0.36	1					
Phantolide	0.30	0.15	1				
Traseolide	0.86	0.38	0.29	1			
Galaxolide	0.55	0.13	0.15	0.74	1		
Tonalide	0.72	0.26	0.23	0.89	0.96	1	
Galaxolidone	0.52	0.09	0.11	0.68	0.98	0.92	1

### Treated Waste Water

Table 4.4.3: Coefficient of determination between polycyclic musk concentrations in treated waste water

	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Galaxolidone
Cashmeran	1						
Celestolide	0.14	1					
Phantolide	0.21	0.98	1				
Traseolide	0.55	0.30	0.28	1			
Galaxolide	0.41	0.79	0.77	0.71	1		
Tonalide	0.04	0.11	0.13	0.01	0.02	1	
Galaxolidone	0.25	0.74	0.71	0.54	0.89	0.01	1



### Correlation Coefficients between Concentration of a Polycyclic Musk Compound in Different Types of Water

Table 4.4.4: Correlation coefficients between concentrations of a polycyclic musk compound in waste water – water after primary treatment, water after primary treatment – treated waste water and waste water – treated waste water

	Waste Water - Water After Primary Settling	Water after Primary Settling - Treated Waste Water	Waste Water - Treated Waste Water
Cashmeran	0.02	-0.11	0.16
Celestolide	0.87	-0.15	-0.15
Phantolide	0.90	0.11	0.10
Traseolide	0.32	-0.52	-0.21
Galaxolide	0.60	-0.63	-0.02
Tonalide	0.51	0.12	0.46
Galaxolidone	0.58	-0.35	0.38

## Appendix 5.1 Polycyclic Musk Flows in the Waste Water Treatment Plant ‚Mittleres Emmental‘

### Waste Water

Table 5.1.1: Polycyclic musk flows in waste water (mg/day)

sample-Nr.	269	276	285	292	299	307	314			
Date	09.04.2002	10.04.2002	11.04.2002	12.04.2002	13.04.2002	21.04.2002	22.04.2002			
Day	Tues	Wed	Thur	Fri	Sat	Sun	Mon	mean	standarddeviation	
Cashmeran	191.31	224.43	245.99	148.84	261.95	89.71	285.66	206.84	68.82	33%
Celestolide	1545.62	2564.38	1724.69	1121.45	1110.38	642.93	1201.56	1415.86	613.01	43%
Phantolide	431.53	396.39	805.60	891.09	499.20	215.79	503.38	534.71	235.98	44%
Traseolide	539.31	549.08	507.04	444.64	643.29	458.27	805.68	563.90	125.30	22%
Galaxolide	36860.69	35276.61	38526.82	28225.69	43603.93	16449.29	53990.52	36133.36	11773.37	33%
Tonalide	11226.23	10887.33	11920.44	9186.68	13409.43	8602.33	15874.81	11586.75	2486.87	21%
Galaxolidone	339.44	290.89	280.14	237.37	389.73	0.00	535.09	296.09	162.87	55%

### Water after Primary Settling

Table 5.1.2: Polycyclic musk flows in water after primary settling (mg/day)

sample-Nr.	270	277	286	293	300	308	315			
Date	09.04.2002	10.04.2002	11.04.2002	12.04.2002	13.04.2002	21.04.2002	22.04.2002			
Day	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	mean	standarddeviation	
Cashmeran	156.42	253.71	189.65	264.95	217.33	188.39	241.96	216.06	39.79	18%
Celestolide	728.56	1971.05	1045.05	1223.50	714.46	465.23	778.85	989.53	497.37	50%
Phantolide	255.87	388.60	547.57	907.45	380.16	212.28	363.12	436.44	233.77	54%
Traseolide	342.76	528.80	420.36	548.63	362.80	364.97	543.59	444.56	92.83	21%
Galaxolide	19536.79	30306.34	29777.18	30871.93	26063.50	21037.28	39422.61	28145.09	6719.49	24%
Tonalide	6753.66	10198.87	9138.00	10424.30	8444.96	6926.50	11771.84	9094.02	1860.09	20%
Galaxolidone	245.39	369.09	348.67	376.48	340.41	256.27	500.24	348.08	85.08	24%

## Treated Waste Water

Table 5.1.3: Polycyclic musk flows in treated waste water (mg/day)

sample-Nr.	271	278	287	294	301	309	316			
	09.04.2002	10.04.2002	11.04.2002	12.04.2002	13.04.2002	21.04.2002	22.04.2002	mean	standarddeviation	
Date	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday			
Day										
Cashmeran	200.33	187.61	108.91	137.93	166.29	93.39	132.80	146.75	39.74	27%
Celestolide	283.67	212.68	199.52	272.32	410.46	145.58	194.35	245.51	86.78	35%
Phantolide	150.36	128.65	111.13	147.28	213.11	83.74	113.28	135.37	41.22	30%
Traseolide	118.02	78.15	69.69	80.46	102.09	64.90	70.53	83.40	19.49	23%
Galaxolide	8321.06	5742.10	4487.89	6558.44	9391.22	4309.32	5163.57	6281.94	1938.82	31%
Tonalide	2671.39	1822.49	1484.20	2082.56	2909.08	3148.62	4004.76	2589.01	865.20	33%
Galaxolidone	382.63	266.19	232.38	318.51	456.20	222.63	303.74	311.76	83.98	27%

## Supernatants from the Thickener

Table 5.1.4: Polycyclic musk flows in supernatants from the thickener (mg/day)

sample-Nr.	273/280/289			296/303		311	318			
	10.- 12.4.2002			13.- 14.4.2002		22.04.2002	23.04.2002	mean	standarddeviation	
Date	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday			
Day										
Cashmeran	0.85	1.30	1.59	0.42	0.42	0.04	0.78	0.77	0.54	70%
Celestolide	9.17	14.03	17.26	5.03	5.03	0.24	3.12	7.70	6.12	80%
Phantolide	3.20	4.89	6.02	2.75	2.75	0.11	1.64	3.05	1.96	64%
Traseolide	4.31	6.59	8.11	1.75	1.75	0.14	2.21	3.55	2.90	82%
Galaxolide	248.25	379.67	467.29	118.20	118.20	9.55	133.28	210.63	162.98	77%
Tonalide	85.80	131.23	161.51	38.69	38.69	3.13	43.05	71.73	57.02	79%
Galaxolidone	2.51	3.85	4.73	1.20	1.20	0.14	1.24	2.13	1.65	78%

### Supernatants from Sludge Storage Tank

Table 5.1.5: Polycyclic musk flows in supernatants from the sludge storage tank (mg/day)

sample-Nr.	275/282/291			298/320	305	313	298/320			
Date	10.4- 12.4.2002			13.4/23.4.2002	16.04.2002	22.04.2002	13.4/23.4.2002			
Day	Wednesday	Thursday	Friday	Saturday	Tuesday	Monday	Tuesday	mean	standarddeviation	
Cashmeran	5.60	5.60	1.68	0.52	4.91	3.88	5.77	3.99	2.10	53%
Celestolide	12.52	12.52	3.76	1.77	16.87	14.53	19.66	11.66	6.60	57%
Phantolide	8.61	8.61	2.58	0.88	9.06	6.79	9.82	6.62	3.50	53%
Traseolide	8.75	8.75	2.62	1.10	10.91	8.69	12.26	7.58	4.15	55%
Galaxolide	585.87	585.87	175.76	60.03	645.75	479.73	667.02	457.15	241.57	53%
Tonalide	185.31	185.31	55.59	21.22	224.88	171.06	235.76	154.16	82.94	54%
Galaxolidone	9.99	9.99	3.00	0.91	10.54	9.34	10.10	7.69	3.98	52%

## Appendix 5.2 Estimation of $K_{oc}$ and $K_{ow}$ Values

	log $K_{ow}$	log $K_{oc}$
Cashmeran	4.50 <sup>1</sup>	3.75 <sup>4</sup>
Celestolide	5.40 <sup>2</sup>	4.47 <sup>4</sup>
Phantolide	5.80 <sup>1</sup>	4.80 <sup>4</sup>
Traseolide	6.30 <sup>6</sup>	5.21 <sup>7</sup>
Galaxolide	5.88 <sup>3</sup>	4.86 <sup>5</sup>
Tonalide	5.77 <sup>3</sup>	4.80 <sup>5</sup>
Galaxolidone	4.79 <sup>7</sup>	3.98 <sup>8</sup>

<sup>1</sup> measured, Eschke, 1999 in Rimkus (1999)

<sup>2</sup> measured, OSPAR Commission (2000)

<sup>3</sup> measured, Eschke, 1994 in Wiegel et al. (2000)

<sup>4</sup> calculated, Wiegel et al. (2000)

<sup>5</sup> calculated, van Plassche et al. (1997)

<sup>6</sup> calculated, Gatermann et al. 1998 [6] and Balk 1998 in [7]

<sup>7</sup> calculated as described by Schwarzenbach et al. (1993), p. 155 (see following paragraph).

<sup>8</sup> calculated, according to the linear relationship established in Figure 5.2.1

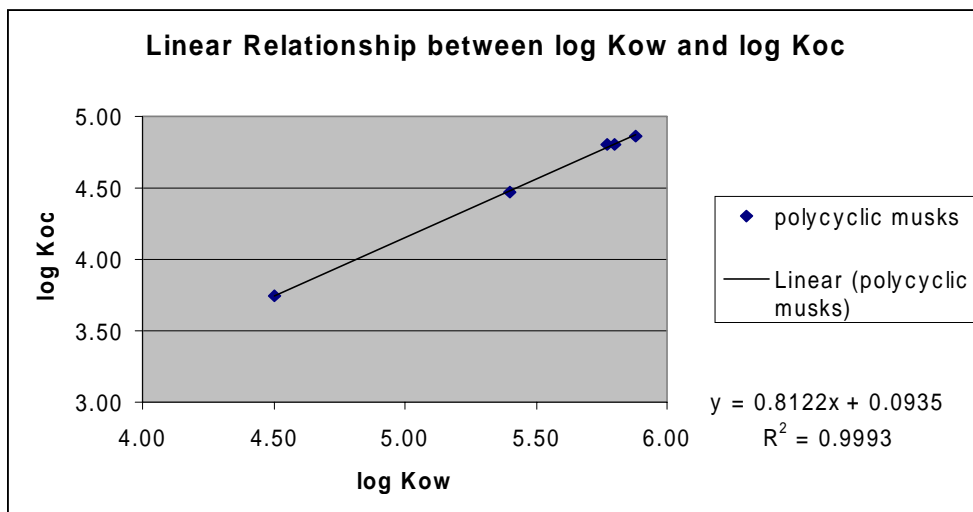


Figure 5.2.1

Linear relationship between  $K_{ow}$  and  $K_{oc}$  values, established from values for Cashmeran, Celestolide, Phantolide, Tonalide and Galaxolide found in the literature

### Estimation of the $K_{ow}$ value for Galaxolidone

$K_{ow}$  for Galaxolidone was derived from the  $K_{ow}$  value of Galaxolide as a structural closely related compound as described by Schwarzenbach et al. (1993), p. 155. The contribution to the  $K_{ow}$  value of the  $\text{CH}_2$  group of Galaxolide was subtracted and the contribution of  $\text{C}=\text{O}$  bound to two aromatic carbons was added.  $(\log K_{ow}(\text{Galaxolidone}) = \log K_{ow}(\text{Galaxolide}) - f_{\text{C aromatic}} - 2 f_{\text{H}}^{\text{Ø}} + f_{\text{(C=O)}}^{\text{ØØ}} = 5.88 - 0.13 - 2 \cdot 0.23 + (-0.5) = 4.79)$

Ø bound to one aromatic carbon

ØØ bound to two aromatic carbons

The  $K_{oc}$  value for Galaxolidone was estimated by the linear relationship established in Figure 5.2.1

### Appendix 5.3 Dry Weight of Different Types of Sludge in the Waste Water Treatment Plant 'Mittleres Emmental'

Sample Nr.	Date	Sample	dw %
272	10.04.2002	boues fraîches eaux surnageantes	3.33
273	10.04.2002	épaississeur	0.41
274	10.04.2002	boues en excès eaux surnageantes	0.73
275	10.04.2002	stockeur	0.33
279	11.04.2002	boues fraîches	2.95
281	11.04.2002	Boues du stockeur	4.53
281	11.04.2002	Boues du stockeur	4.67
283	11.04.2002	Boues après hygiénisation	2.90
284	11.04.2002	boues en excès	0.67
288	12.04.2002	boues fraîches eaux surnageantes	
289	12.04.2002	épaississeur	
290	12.04.2002	boues en excès eaux surnageantes	
291	12.04.2002	stockeur	
295	13.04.2002	boues fraîches eaux surnageantes	2.86
296	13.04.2002	épaississeur	0.19
297	13.04.2002	boues en excès eaux surnageantes	0.65
298	13.04.2002	stockeur	0.14
302	14.04.2002	boues fraîches eaux surnageantes	3
303	14.04.2002	épaississeur	0.16
304	14.04.2002	boues en excès eaux surnageantes	0.74
305	16.04.2002	stockeur	0.17
306	16.04.2002	boues du digesteur	2.15
310	22.04.2002	boues fraîches eaux surnageantes	4.32
311	22.04.2002	épaississeur	0.26
312	22.04.2002	boues en excès eaux surnageantes	0.76
313	22.04.2002	stockeur	0.16
317	23.04.2002	boues fraîches eaux surnageantes	3.25
318	23.04.2002	épaississeur	0.22
319	23.04.2002	boues en excès eaux surnageantes	0.73
320	23.04.2002	stockeur	0.19
	mean	boues fraîches	3.29
	mean	boues en excès	0.71

Dry weight was determined by weighting the sample before and after dehydration, which was accomplished by the FAL (Eidgenössische Forschungsanstalt für Agrarökologie und Landbau, Liebefeld (BE))

## Appendix 5.4 Expected Polycyclic Musk Concentrations in Primary Sludge calculated from the $K_{oc}$ Values

### Expected Primary Sludge Concentration ( $\mu\text{g}/\text{kg dw}$ )

Table 5.4.1: Expected polycyclic musk concentrations ( $\mu\text{g}/\text{kg dw}$ ) in primary sludge, calculated for the primary settling tank

	09.04.2002 Tuesday	10.04.2002 Wednesday	11.04.2002 Thursday	12.04.2002 Friday	13.04.2002 Saturday	21.04.2002 Sunday	22.04.2002 Monday	mean
Cashmeran	987	1'830	1'243	1'938	1'263	1'039	1'586	1'412
Celestolide	24'114	74'633	35'959	46'957	21'797	13'468	26'784	34'816
Phantolide	18'106	31'459	40'282	74'459	24'796	13'138	26'697	32'705
Traseolide	62'343	110'033	79'485	115'710	60'825	58'060	102'729	84'169
Galaxolide	1'587'274	2'816'869	2'515'056	2'908'420	1'951'857	1'494'890	3'327'854	2'371'746
Tonalide	477'901	825'630	672'226	855'343	550'824	428'680	865'493	668'014
Galaxolidone	2'628	4'522	3'882	4'676	3'361	2'401	5'567	3'862

### Expected Primary Sludge Flow (mg/cap/year)

Table 5.4.2: Expected polycyclic musk concentrations (mg/cap/year) in primary sludge, calculated for the primary settling tank

	09.04.2002 Tuesday	10.04.2002 Wednesday	11.04.2002 Thursday	12.04.2002 Friday	13.04.2002 Saturday	21.04.2002 Sunday	22.04.2002 Monday	mean
Cashmeran	78	125	20	132	84	96	99	91
Celestolide	1'918	5'102	587	3'204	1'450	1'248	1'678	2'170
Phantolide	1'440	2'150	658	5'081	1'649	1'217	1'673	1'981
Traseolide	4'958	7'521	1'297	7'896	4'046	5'380	6'438	5'362
Galaxolide	126'227	192'549	41'055	198'477	129'840	138'527	208'540	147'888
Tonalide	38'005	56'436	10'973	58'371	36'642	39'725	54'236	42'055
Galaxolidone	209	309	63	319	224	222	349	242

### Expected Primary Sludge Concentration ( $\mu\text{g}/\text{kg dw}$ )

Table 5.4.3: Expected polycyclic musk concentrations ( $\mu\text{g}/\text{kg dw}$ ) in primary sludge, calculated for the thickener

	09.04.2002 Tuesday	10.04.2002 Wednesday	11.04.2002 Thursday	12.04.2002 Friday	13.04.2002 Saturday	21.04.2002 Sunday	22.04.2002 Monday	mean
Cashmeran	2'522	2'845	2'558	1'778	1'695	1'602	2'904	2'272
Celestolide	143'316	161'680	145'350	111'158	105'970	48'478	60'640	110'942
Phantolide	106'826	120'514	108'342	130'029	123'961	46'183	68'181	100'577
Traseolide	369'835	417'224	375'084	212'891	202'956	158'882	235'911	281'826
Galaxolide	9'522'365	10'742'516	9'657'508	6'415'887	6'116'479	4'804'216	6'365'904	7'660'697
Tonalide	2'866'529	3'233'833	2'907'211	1'829'162	1'743'801	1'370'341	1'791'067	2'248'849
Galaxolidone	12'717	14'346	12'897	8'599	8'197	9'294	7'807	10'551

### Expected Primary Sludge Flow (mg/cap/year)

Table 5.4.4: Expected polycyclic musk loads (mg/cap/year) in primary sludge, calculated for the thickener

	09.04.2002 Tuesday	10.04.2002 Wednesday	11.04.2002 Thursday	12.04.2002 Friday	13.04.2002 Saturday	21.04.2002 Sunday	22.04.2002 Monday	mean
Cashmeran	178	160	140	82	96	125	180	137
Celestolide	10'131	9'095	7'943	5'134	5'977	3'786	3'753	6'545
Phantolide	7'551	6'779	5'921	6'005	6'991	3'606	4'219	5'868
Traseolide	26'143	23'469	20'498	9'832	11'446	12'407	14'599	16'914
Galaxolide	673'119	604'277	527'786	296'312	344'961	375'163	393'933	459'364
Tonalide	202'630	181'906	158'880	84'478	98'348	107'010	110'834	134'870
Galaxolidone	899	807	705	397	462	726	483	640



### Expected Activated Sludge Concentration ( $\mu\text{g}/\text{kg dw}$ )

Table 5.4.5: Expected polycyclic musk concentrations ( $\mu\text{g}/\text{kg dw}$ ) in activated sludge

	09.04.2002 Tuesday	10.04.2002 Wednesday	11.04.2002 Thursday	12.04.2002 Friday	13.04.2002 Saturday	21.04.2002 Sunday	22.04.2002 Monday	mean
Cashmeran	5'564	9'973	7'069	10'514	6'317	7'285	8'706	7'918
Celestolide	136'004	406'638	204'425	254'819	108'985	94'414	147'066	193'193
Phantolide	102'119	171'403	229'001	404'063	123'981	92'104	146'590	181'323
Traseolide	351'622	599'515	451'874	627'921	304'124	407'030	564'071	472'308
Galaxolide	8'952'370	15'347'734	14'298'084	15'783'025	9'759'284	10'479'963	18'272'806	13'270'467
Tonalide	2'695'406	4'498'450	3'821'604	4'641'662	2'754'120	3'005'272	4'752'305	3'738'403
Galaxolidone	14'823	24'640	22'070	25'373	16'803	16'829	30'566	21'586

### Expected Activated Sludge Flow (mg/cap/year)

Table 5.4.6: Expected polycyclic musk loads (mg/cap/year) in primary activated sludge

	09.04.2002 Tuesday	10.04.2002 Wednesday	11.04.2002 Thursday	12.04.2002 Friday	13.04.2002 Saturday	21.04.2002 Sunday	22.04.2002 Monday	mean
Cashmeran	8'919	14'698	11'022	14'861	14'207	10'223	13'647	12'511
Celestolide	218'018	599'264	318'732	360'164	245'112	132'498	230'532	300'617
Phantolide	163'699	252'597	357'051	571'107	278'839	129'255	229'786	283'191
Traseolide	563'657	883'508	704'548	887'510	683'990	571'210	884'207	739'805
Galaxolide	14'350'832	22'618'023	22'293'116	22'307'900	21'949'116	14'707'199	28'643'466	20'981'379
Tonalide	4'320'790	6'629'386	5'958'523	6'560'575	6'194'153	4'217'490	7'449'457	5'904'339
Galaxolidone	23'762	36'312	34'411	35'862	37'791	18'167	47'914	33'460