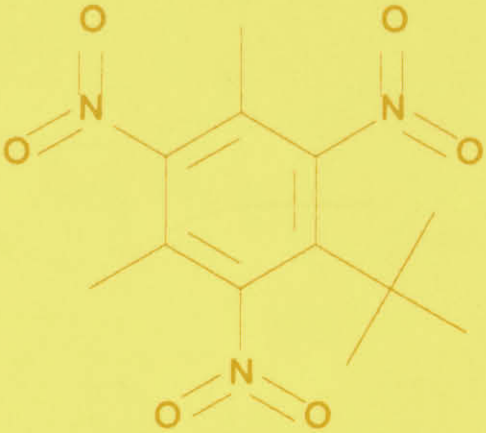
ASSOCIATION OF RIVER WATERWORKS

RIWA

March 2000

Fragrance Ingredients



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PREFACE

At the initiative of the RIWA, in the project group “Studies on substances” survey documents are being drafted for chemical substances and groups of substances that may be of importance with regard to the production of drinking water. Issues important at drinking water plants mainly relate to nature, magnitude and (eco)toxicological meaning of the group of substances as a whole, or of the most important representatives thereof, trends in the use and the meaning of the group to the production of drinking water from surface water.

An assignment for the drafting of a survey document on the group of fragrances as submitted in the quotation number MI031001/4605N of July 8, 1996 was given to BKH Consulting Engineers on December 19, 1996. Project leader on behalf of the RIWA project was Dr. P.G. Stoks of the WRK (Water Transport Company Rhine-Kennemerland Ltd.) at Nieuwegein.

For this survey document data were available in public scientific literature, but especially use was made of recent reports by RIFM (Research Institute of Fragrance Materials, USA), RIVM (National Institute of Public Health and Environmental Protection), RIZA (Institute for Inland Water Management and Wastewater Treatment), RIVO (National Fisheries Research Institute), Dutch Consumers´ Association and BKH Consulting Engineers, and of personal communications of employees of RIZA and RITOX (Research Institute of Toxicology), RIFM (Research Intstitute of Fragrance Materials) and NEA (Netherlands Association of Fragrances and Flavours Manufacturers).

SUMMARY

Fragrances are used in cosmetic products and in detergents. During the past few years some ingredients of fragrances have become of interest, since residues have been found in samples of surface water, fish, human adipose tissue and human milk. The wider interest for fragrances also gave rise to the producers of drinking water to investigate the risks of fragrances in more detail.
Eight substances, viz. the nitromusks musk ketone and musk xylene, the polycyclic musks AHTN, HHCB, ADBI, AITI and AHMI, and acetyl cedrene have at some time been detected in surface water, and limonene has been found in the effluent of a waste water purification plant. For these substances the physical-chemical properties and the toxicity data have been compiled. The analytical and detection methods have been summarized and an overview is given of the concentrations found in the environment.

The risk of these fragrance ingredients to man is assessed by comparing the toxicity with the exposure. The direct exposure to the most important ingredients, AHTN and HHCB, for example as a consequence of applying perfumes with these ingredients onto the skin, is estimated to be many times below the level where effects are expected. A risk assessment of indirect exposure as a consequence of drinking water containing fragrances ingredients should be based on measured concentrations in drinking water. Since these data were not available, a worst case situation was assumed: the use of unpurified surface water as drinking water. It was assumed that a person weighing 70 kg drinks 2 litres of water per day. In that case the exposure to these fragrance ingredients is estimated to be at least a factor $5*10^6$ below the level where systemic effects are expected. The safety margin for the nitromusks musk ketone and musk xylene, and for the other polycyclic musks, is even much larger. When, as a compensation for the short duration of the available toxicity test, a very high safety factor is applied to acetyl cedrene (100.000), the safety margin is still more than 1600.
It is concluded that the risks of fragrance ingredients to man by the intake through drinking water is very small. Taste and odour tests with HHCB indicate that this substance will not be tasted or smelled in samples from Dutch surface water.

The risk to aquatic organisms is assessed by comparing the toxicity with concentrations found in Dutch surface water. The risk of fragrances to aquatic organisms is shown to be very small.

Based on the environmental risk assessments of musk ketone, musk xylene, AHTN and HHCB carried out by RIVM, the Minister of VROM (Ministry of Housing, Spatial Planning and the Environment) has concluded that there is no reason for concern or to take measures. Industry expects that the use of nitromusks and polycyclic musks will stabilise at a lower level. The Minister of VROM has requested producers to report the volumes of use of AHTN and HHCB in Europe on a voluntary basis.

1. INTRODUCTION

The production and use of fragrances dates back to the previous century and found its origin in the isolation of aromatic chemicals from essential oils and natural extracts. A new branch of industry came into being and specialised in combining and mixing aromatic chemicals to make products having a characteristic odour. In the sixties the industry knew an enormous growth because of the ever increasing demand for products with fragrances. Of the thousands of aromatic chemicals, about 3000 are being used in the fragrance industry. Three to four hundred of these 3000 substances are of natural origin.

The fragrance industry covers three sectors:
1 essential oils and natural extracts;
2 aromatic chemicals;
3 compound products.

Essential oils and natural extracts are complex aromatic mixtures of animal or herbal origin which are obtained by distillation, pressing, solvent extraction or maceration. The complex aromatic mixtures can be used to add to consumer products, or to serve as raw material in compound products. It is also possible to use the mixtures as a source for isolated aromatic chemicals. Aromatic chemicals consist of organic substances having an unambiguous chemical structure. They have been isolated from essential oils and natural extracts or they have been synthesized. Aromatic chemicals are used to make compound products. Compound products consist of a mixture of various complex aromatic mixtures of essential oils and natural extracts, or of mixtures of aromatic chemicals [Somogyi, 1996].
Fragrances are mainly used in so-called perfume[1] extracts and cosmetics (eau de toilette, creams etc.), in domestic products (fabric softeners, detergents) and in soaps. The concentration of fragrances varies strongly per product. In perfume extracts the concentration may increase to 20% and in body lotion and eau de toilette the concentration varies from 5% to 8%. The average concentration in cosmetics is 0.5% and in products in which they are meant to mask odours it is less than 0.1%. For detergents the concentration ranges from 0.1% for some liquid products to 5 % for some products for cleaning bathrooms [Groot and Frosch, 1997; Colipa, 1995]. The fragrances occurring most frequent in Dutch cosmetic products are linalool (in 91% of the products), phenyl ethyl alcohol (79%), benzyl acetate (78%), citronellol (71%) and limonene (71%) [Groot and Frosch, 1997].

Fragrances have attracted attention recently, since some of these substances have been detected in samples of surface water, fish and human adipose tissue and human milk [Rimkus and Wolf, 1993ab, 1995, 1996; Rimkus et al., 1994; Rimkus and Brunn, 1997; Brunn and Rimkus, 1997; Eschke et al., 1994a,b; Eschke et al., 1995a,b; Müller et al., 1996; Liebl and Ehrenstorfer, 1993; Hahn, 1993; Breukel and Balk, 1996]. In the Netherlands this has led to an investigation by the Consumentenbond into the occurrence of so-called nitromusks in consumers products such as cosmetics and detergents, and in eel [Consumentenbond, 1995, 1996].

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[1] Concentrated mixture which consists of a solution of a perfume composition (20%) in alcohol. Eau de toilette, after shave, eau de Cologne and body lotion are dilutions thereof.

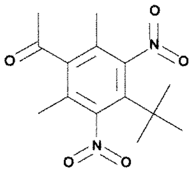
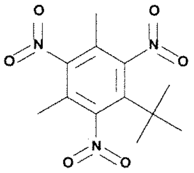
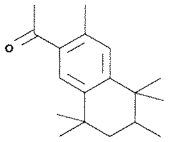
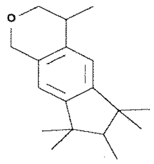
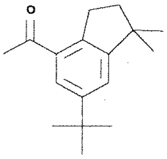
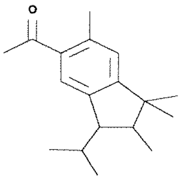
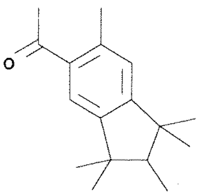
In 1996 the Consumentenbond again reported on fragrances in products. In washing powders nitromusks were no longer found, but rather the substitutes, namely polycyclic musks. In 1997 the Consumentenbond reported that polycyclic musks are also applied in cosmetics such as deodorant [Consumentenbond, 1997].

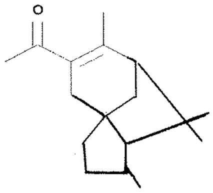
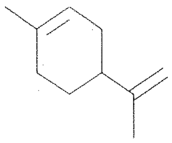
As a result of the first investigation by the Consumentenbond the Ministries of VROM (Housing, Spatial Planning and Environment) and VWS (Transport, Public Works and Water Management) were asked to evaluate the use of nitromusks and to take measures to decrease both the distribution in the environment and the exposure of humans. At the request of VROM/DGM (Directorate-General for Environmental Protection) an environmental risk assessment was carried out in 1996 by RIVM for two of the most widely used nitromusks. This risk assessment was carried out in co-operation with NEA (Dutch Association of Fragrance and Flavour Producers) [Tas and van der Plassche, 1996, Tas et al., 1997]. The human health effects are judged within the scope of the European Directive for Cosmetics (EC Scientific Committee on Cosmetics). In the mean time the two nitromusks, musk ketone and musk xylene, have been put on the so-called Third Priority List of the EC within the scope of the Existing Chemicals Regulation (EEC 793/93).This implies that the risks of these substances to the environment and to public and occupational health will be evaluated once more.
Simultaneously with the environmental risk assessment of the nitromusks an environmental risk assessment was carried out by the Research Institute of Fragrance Materials (RIFM) in co-operation with NEA and BKH Consulting Engineers for two representatives of the polycyclic musks, AHTN and HHCB. In the mean time this risk assessment has been adopted by RIVM [Van de Plassche and Balk, 1997] and published [Balk and Ford 1999a,b].

Besides the four substances mentioned above, musk ketone, musk xylene, AHTN and HHCB, traces of other fragrances are also detected in Dutch surface water. According to RIZA, the polycyclic musks AITI and AHMI are found as well and furthermore acetyl cedrene (not belonging to the musks). RITOX has found limonene in effluent samples. In a Swiss river yet another polycyclic musk was found, namely ADBI. For these substances found in water samples, as far as known, no risk assessments have been carried out yet. In table 1 names, structures and other data on these nine selected substances are given.

In this report the significance of these nine substances for the production of drinking water from surface water, will be investigated.

Table 1 Fragrances

Identification and characteristics		Structure
Musk ketone	CAS: 81-14-1 $C_{14}H_{18}N_2O_5$, MW 294.3 3,5-dinitro-2,6-dimethyl-4-t-butyl-acetofenone	
Musk xylene	$C_{12}H_{15}N_3O_6$, MW 297.3 2,4,6-trinitro-1,3-dimethyl-5-t-butylbenzene	
AHTN Tonalid®, Fixolide®	CAS: 21145-77-7/1506-02-1 $C_{18}H_{26}O$, MW 258.4 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphtalene 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphtyl)ethan-1-one 6-acetyl-1,1,2,4,4,7-hexamethyl-tetralin	
HHCB Galaxolide 50®, Abbalide®	CAS: 1222-05-5 $C_{18}H_{26}O$, MW 258.4 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexa-methylcyclopenta[gamma]-2-benzopyran	
ABDI Celestolide®, Crysolide®	CAS: 13171-00-1 $C_{17}H_{24}O$, MW 244.4 4-acetyl-6-tert-butyl-1,1-dimethylindan	
AITI Traseolide®	CAS: 68140-48-7 $C_{18}H_{26}O$, MW 258.4 5-acetyl-1,1,2,6-tetramethyl-3-isopropyl-indan	
AHMI Phantolid®	CAS: 15323-35-0 $C_{17}H_{24}O$, MW 244.4 5-acetyl-1,1,2,3,3,6-hexamethylindan	

Identification and characteristics		Structure
Acetyl cedrene	CAS: 32388-55-9 $C_{17}H_{26}O$, MW 246.4	
Limonene	CAS: 5989-54-8 $C_{10}H_{16}O$, MW 136.2	

2. SOURCES AND USE

Sources

Nitromusks and polycyclic musks are widely used ingredients in fragrance mixtures for in cosmetics such as eau de parfum, deodorant, shampoo, creamwash, soap, bath and shower foam, and for detergents, fabric softeners, cleaning products and air fresheners. The most important application by far is in detergents and cleaning products. The odour suggests natural musk, a strong smelling substance from a gland of male musk deer and musk rats. In the consumer products mentioned, synthetic musks form an important component, not only because of their unique odour but also because they intensify the odour of fragrance mixtures and prolong their effect, and because of their ability to bind fragrances to fabric and other surfaces.
The result of the application in personal care products, such as shampoo and soap and in detergents and cleaning products, is that the used volume is for the greater part discharged with the waste water to the sewer to waste water treatment plants. Since these products are used daily, there is a continuous domestic emission to the sewer.

Acetyl cedrene is a product made from cedar wood oil. Again this is mainly applied in detergents and cleaning products.

Limonene occurs naturally (δ-limonene) in food-stuffs, its most important source being oranges and other citrus products. The substance is also made synthetically (racemic mixture). Limonene is also used as solvent for the removal of, for instance, oil, but it is also added to soap and cleaning products, lending it the “fresh scent of lime”. The fraction applied in soap and cleaning products ultimately ends up in the waste water purification plant.

Consumption quantities

In connection to the environmental risk assessments [Tas and van de Plassche, 1996; Van de Plassche and Balk, 1997] an attempt has been made to estimate the use of musk ketone, musk xylene, AHTN and HHCB. This estimation is based on inquiries among the producers and formulators of fragrance ingredients by RIFM for the years 1992 and 1995. The data relate to the formulated volume[2] in entire Europe, including Eastern Europe, and has not been corrected for export out of Europe.

Subsequently it was assumed that the total volume was marketed only in the EC and was consumed by its 365 million inhabitants. Because of this the estimated consumption per person, as represented in table 2, is an overestimation. Assuming that the consumption per person is the same all over the EC, an indication can be obtained of the consumption of these musk fragrance ingredients in the river-basins of the Rhine and the Meuse. The use of acetyl cedrene and limonene is in the same order of magnitude as that of the polycyclic musks [Ford, personal comm.].

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[2] Producers of fragrances often do not know in which perfume compositions their fragrances are applied, nor in which consumer products and brands they are marketed.

Table 2 Estimation of the daily consumption (mg) per person of musk fragrances (RIFM data, 1997, see Van de Plassche and Balk, 1997).
See the text for the explanation on the correctness of the numbers

	1995
	max. consumption in mg per person per day
musk ketone	0.5
musk xylene	0.8
AHTN	4.4
HHCB	11

3. ENVIRONMENTAL BEHAVIOUR

In table 3 the main characteristics of the various fragrance ingredients are given which define the behaviour of the substances in the environment. The octanol-water distribution coefficients (logKow) of musk ketone, musk xylene, AHTN and HHCB have been measured. As the measured log Kow for the polycyclic musks AHTN and HHCB turn out to be much lower than the estimations, it is expected that the estimations for the related substances ADBI, AITI and AHMI are too high as well. The solubility in water has been estimated for all substances on the basis of logKow; for ADBI, AITI and AHMI the estimated solubility in water is expected to be too low as a result of the overrated estimation of logKow.

Table 3 Characteristics of fragrances
S: solubility in water,
logKow: octanol/water distribution co-efficient (measure for the solubility of lipid fractions)
BCF: bioconcentration factor in fish

Substance	Characteristics	
Musk ketone	S = 1.9 mg/l[s], 0.46 mg/l[n]	LogKow = 4.3 [m], 3.8 [n] BCF = 1380 (radio-label)
Musk xylene	S = 0.49 mg/l[s], 0.15 mg/l[n]	LogKow = 4.9 [m], 4.4 [n] BCF = 1600 (radio-label)
AHTN	S = 0.36 mg/l[n], 1.25 mg/l[m]	LogKow = 5.7 [m], [6.4 [s]] BCF = 597 (parent material)
HHCB	S = 0.19 mg/l[n], 1.75 mg/l[m]	LogKow = 5.9 [m], [6.3 [s]] BCF = 1584 (parent material)
ADBI	S = 0.22 mg/l[s]	LogKow = 5.9 [s]
AITI	S = 0.09 mg/l[s]	LogKow = 6.3 [s]
AHMI	S = 0.25 mg/l[s]	LogKow = 5.85 [s]
Acetyl cedrene	S = 0.65 mg/l[s]	LogKow = 5.36 [s]
Limonene	S = 3.2 mg/l[s]	LogKow = 4.57 [s]

s estimated [SRC, 1996]
m measured [Schramm et al., 1996]
n measured [in Van de Plassche and Balk, 1997]

The common characteristics of the substances are: reasonably to strongly lipophilic and poorly soluble in water, at a level of approximately 1 mg/l.

The biodegradability of musk ketone, musk xylene, AHTN and HHCB has been investigated in standard tests. These tests measure the consumption of oxygen or the production of carbon dioxide. Under the conditions of the standard tests the substances did not mineralise. The substances, however, are not persistent and degradation products do arise. An investigation was performed into AHTN and HHCB with cultures of micro-organisms from soil. It was shown that these two substances can degrade to more polar metabolites

within a few days to a week. AHTN, HHCB and musk ketone are also known to be metabolised by fish to more polar degradation products within one to three days [Balk and Ford 1999a,Van de Plassche and Balk, 1997; Tas et al., 1997]. Recently degradation products of musk keton and musk xylene were found in a sewage treatment plant effluent. These products, amino-derivatives, imply that a reduction of the molecules has taken place during the treatment process [Gatermann et al, 1998].
The biodegradability of the other substances in the table is not known, however an estimation can be made based on the structure fragments. The estimated biodegradation rates for the polycyclic musks ADBI, AITI and AHMI are similar to those for AHTN and HHCB. The biodegradability of acetyl cedrene falls in that class as well. The degradation (mineralisation) of limonene passes at a much higher rate, in the order of days to weeks [SRC, 1996, Biodegradation].

Based on the lipophility of the substances it is to be expected that the substances adsorb strongly to organic material such as sewage sludge, suspended matter and sediment, and accumulate in biological matter.

AHTN and HHCB have indeed been detected in sewage sludge and river sediment [Blok, 1997; Eschke, 1996] and AHTN, HHCB and musk xylene have been found in suspended matter of the Rhine and the Meuse [Breukel and Balk, 1996].
For musk ketone and musk xylene the bioconcentration (BCF) factor seems reasonably predictable based on logKow, but in this respect it should be pointed out that the BCF in the investigation has been calculated for the radio-active residue in the fish. That means that also radio-active metabolites are included and that the actual BCF (based on the parent material) is somewhat lower. From the results of the bioaccumulation experiments with musk ketone it can be concluded that the substances in fish are metabolised at a relative conversion of 0.3 to 1 per day, related to the amount present in the fish. (This means that 3 to 10 days after cessation of exposure more than 90% of the substance in fish will have been converted.)
For AHTN and HHCB it appears that the BCF is considerably lower than predicted on the basis of logKow. Also these two substances are converted to polar metabolites with a relative conversion of 0.3 per day. These polar metabolites are quickly excreted [Tas et al., 1997; Balk and Ford, 1999a].

4. ANALYSIS AND DETECTION METHODS

Analysis methods have been developed and described by a great number of researchers, such as Eschke et al. [1994a], Rimkus and Wolf [1993a, 1995] and Müller et al. [1996] for the nitromusks and Eschke et al. [1994b, 1995a] for the polycyclic musks. A summary of methods applied by various researchers is given in Tas et al. [1996] for nitromusks and in Balk and Ford [1999a] for polycyclic musks.

The analysis of fragrances such as nitromusks and polycyclic musks is preceded by extraction. In water samples the substances can be concentrated with C_{18}- or XAD solid phase and/or extracted with the aid of organic solvents. Water samples which contain suspended matter, such as surface water or waste water, can be extracted directly with n-hexane or other organic solvents. For the separation and the detection of nitromusks use has been made of GC-ECD, but also of GC-MS. For the polycyclic musks GC-MS is always applied. Some of these substances have the same chromatogram (for instance ADBI and AHMI; AHTN, HHCB and ATTN). Also the mass spectra of these substances look alike (for instance AHTN and ATTN; ADBI and AHMI), which might cause problems for the separation with GC-MS. The detection limits vary from 1 to 30 ng/l water starting from water samples of 10 to 2 litres. Freeze-dried samples with suspended matter can be boiled (soxtec)[3] and extracted with hexane/acetone (detection limit 0.05 mg/kg). For the extraction of samples of sewage sludge extraction with dichloromethane was applied (reporting limit 1 mg/kg).
For the extraction from fish soxhlet[3] extraction with, for instance, hexane or methanol was applied, followed by a clean-up with for instance Gel Permeation Chromatography (GPC) and/or adsorption chromatography on silica gel. With these techniques the detection limits are 1 to 10 μg/kg fresh weight and 10 to 20 μg/kg fat (starting from a sample of 10 g).

A new development is the so-called biomimetic extraction on an Empore disk. This is a solid phase extraction, during one or two weeks, and no exhaustion of the sample liquid takes place. The disk is directly injected into a GC-MS system [Verbruggen, 1997]. The concentrations are estimated with the aid of various correction factors. The lowest concentration reported is 0.6 ng/l.

The remaining polycyclic musks, acetyl cedrene, limonene and other organic fragrances have comparable chemical properties and can, in principle, be measured using the same analytical techniques.

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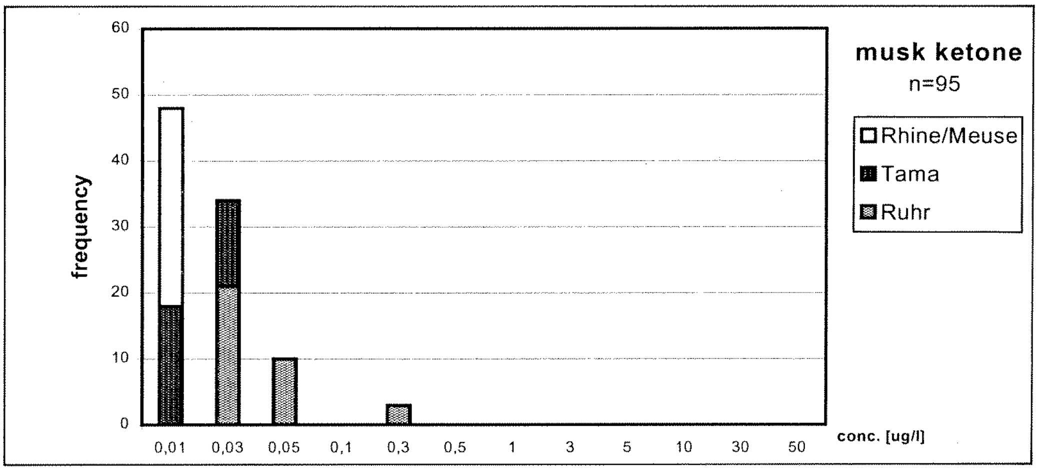
[3] Soxtec and soxhlet are very similar extraction techniques; they differ amongst others in the glassware and the method of heating of the solvent.

5. CONCENTRATIONS IN THE ENVIRONMENT

Nitromusks

During measurements by RIZA in the large rivers in The Netherlands, musk ketone and musk xylene were hardly found in concentrations above the detection level. At all times the concentrations in water were below the detection level of 0.01 μg/l (35 samples in the Meuse at Eijsden and 32 samples in the Rhine at Lobith from 1994 to 1996). In suspended matter musk xylene was detected only three times in samples from the Meuse from 1990 and 1994 (28 samples of the Rhine and the Meuse between 1990 and 1996). In the Ruhr and the Lauchert in Germany and the Tama in Japan, however, higher levels in samples of surface water were found, see figure 1. Concentrations in the eastern part of the North Sea were below the detection limit (0.02 ng/l) for musk ketone and around the detection limit for musk xylene [Gaterman et al., 1995]. These concentrations are three orders of magnitude lower than in the Rhine and the Meuse.



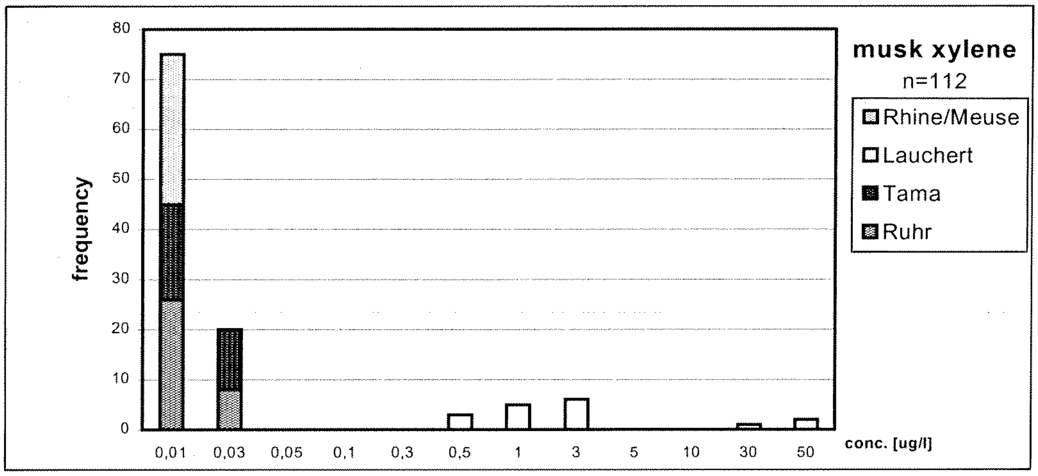


Figure 1. Concentrations of musk ketone and musk xylene in water samples
Ruhr [Eschke et al., 1994], Tama [Yamagashi, 1983], Lauchert [Hahn, 1993], Rhine/Meuse [Breukel and Balk, 1996]

Musk ketone and musk xylene were detected only occasionally in sewage treatment influents in The Netherlands (≤0.2 µg/l) [Rijs and Schäfer 1998]. During the treatment process the substances are adsorbed to the sludge. Concentrations in effluents discharged into the Ruhr in Germany the median concentrations were 0.8 µg/l for musk ketone and 0.1 µg/l for musk xylene [Eschke et al., 1994]. For the effluent of the three largest purification plants in Sweden relatively high effluent concentrations between 1 and 5 µg/l were reported [Paxéus, 1996].
In The Netherlands two sampling programmes have been carried out to investigate the contents in fish. In 1995 the median concentrations of musk ketone and musk xylene in the liver of cod from the southern North Sea were < 0.004 mg/kg fresh weight. In eel from rivers and lakes the median concentrations were 0.02 mg/kg for musk ketone and 0.03 mg/kg for musk xylene. In the IJsselmeer the concentrations were lower than in the large rivers. The concentrations of other nitromusks such as musk ambrette, musk moskene and musk tibetene were all below the detection limit [Wiertz, 1995]. In a second series of samples from 1996 the concentrations in eel were lower by a factor 2 or more. Both in pike-perch and freshwater consumption fish, and in mussels and shrimps from the coastal area the concentrations were below the detection limit (0.5 to 1 µg/kg fresh weight) [de Boer and Wester, 1996].
in Germany the amino-metabolites of musk ketone and musk xylene were detected in effluent at a level of 0.25 µg/l and 0.04 µg/l, respectively [Gatermann et al., 1998]. These figures represent 50% of the influent concentrations of the musk ketone and 25% for musk xylene, whereas the parent material was reduced by 99% and 93%, respectively. Concentrations in fish were around a level of 50 $\mu g/kg_{lipid}$ for musk xylene and its amino-metabolites. The concentrations of musk ketone in fish were of the same order of magnitude, but its amino-metabolite was lower by an order of magnitude [Rimkus et al. 1998 in BUWAL 1999] .

Polycyclic musks

Concentrations in the large Dutch rivers have been measured by RIZA since 1994. In the period between 1994 and 1996 median concentrations of AHTN and HHCB in the Rhine (Lobith) and Maas (Eijsden) ranged between 0.05 and 0.08 µg/l. The 90-percentile values for both rivers are 0.11 µg/l for AHTN and 0.19 µg/l for HHCB [Breukel and Balk 1996]. Median concentrations in other Dutch surface water samples of 1997 were lower, 0.04 µg/l for AHTN and 0.01 µg/l for HHCB [recalculated from suspended matter concentrations of Rijs and Schäfer 1998].
Median concentrations in suspended matter for AHTN were 0.24 and 0.84 mg/kg dw in the Rhine and the Meuse, respectively, and for HHCB 0.06 and 0.20 mg/kg, respectively [Breukel and Balk, 1996]. Suspended matter concentrations in other Dutch surface waters were 0.12 mg/kg dw for AHTN and 0.05 mg/kg dw for HHCB.

With biomimetic extraction on Empore disks the estimated concentrations varied between 1 ng/l for AHTN and 2.7 ng/l for HHCB in four relatively clean areas in The Netherlands. In some other areas (for instance the Scheldt and the Meuse at Maastricht) the concentrations were at the level of sewage treatment effluents, i.e. the dilution factor of effluent to surface water is very low [Verbruggen, 1997]. The concentrations in effluent varied between 0.11 and 0.42 µg/l for both substances.

Median concentrations in water of the Ruhr in Germany are 0.2 μg/l for AHTN and 0.5 μg/l for HHCB (23 samples). In effluents which are discharged into the Ruhr, the median concentrations were 1.6 and 1.2 μg/l for AHTN and HHCB, respectively (21 samples) [Eschke et al., 1995a].

In the meantime, more authors have measured concentrations of these and other polycyclic musks. An overview of measured concentrations in water is given in figure 2.

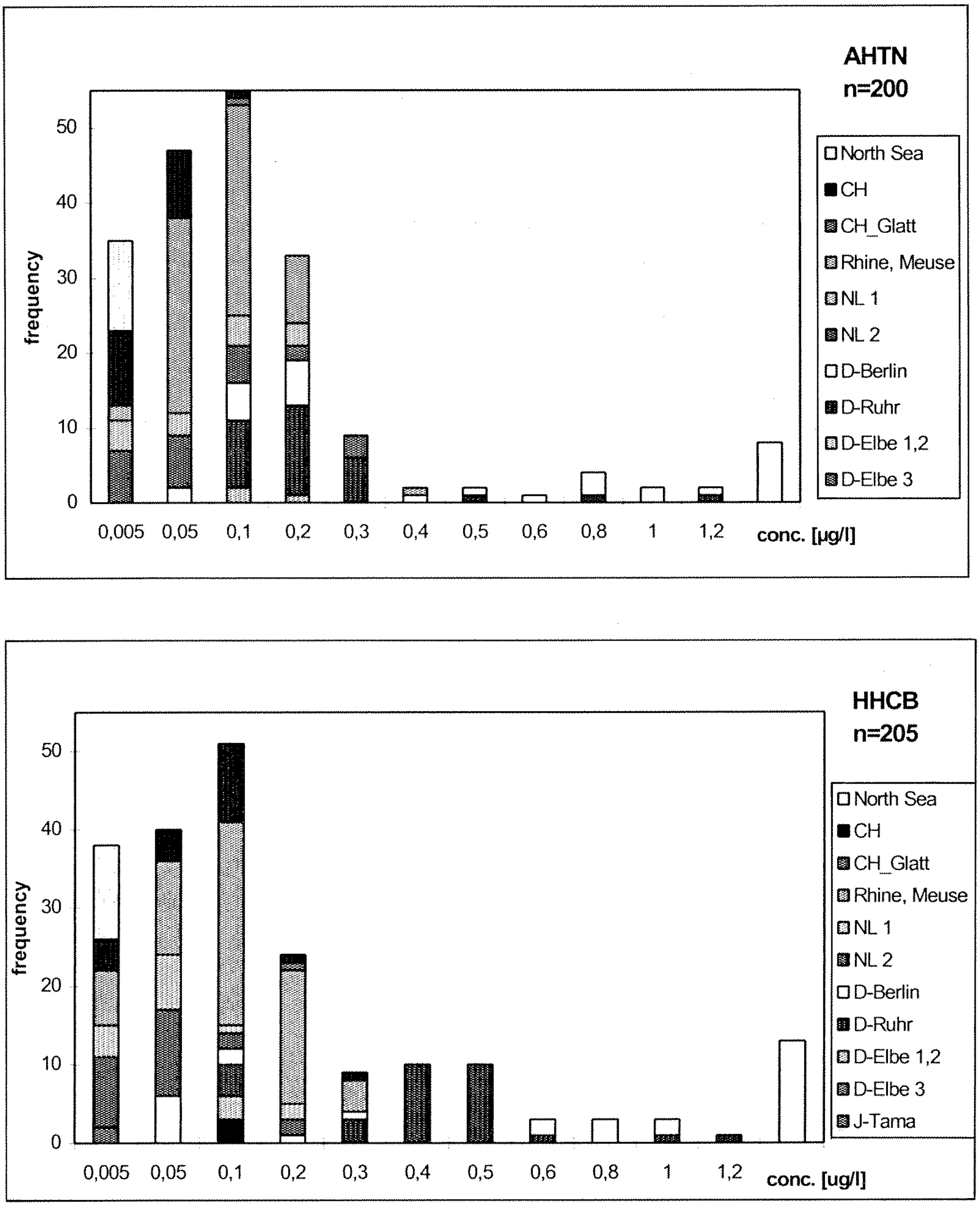


Figure 2. *Concentrations in surface water samples*
[from Balk and Ford 1999a]

Concentrations in eel from the main Dutch rivers contained the highest levels of AHTN and HHCB, up to 0.1 mg/kg fresh weight [Rijs and Schäfer 1998]. Median concentrations in eel in the Ruhr were slightly higher. Because of the lower fat content, levels in whitefish in the Ruhr were lower: 0.029 and 0.018 mg/kg fresh weight for AHTN and HHCB, respectively [Eschke et al., 1995a].

The presence of other polycyclic musks is reported now and again. In the Meuse and the Rhine AITI is found regularly in concentrations of approximately 0.02 µg/l and there are indications for the presence of AHMI [Breukel and Balk, 1996]. In the Glatt (Switzerland) ADBI is found in a concentration of 3 ng/l (lower than AHTN by a factor of 25, lower than HHCB by a factor of 40) [Müller et al., 1996]. In the effluent of sewage treatment plants along the Ruhr the median concentration of ADBI was lower than AHTN by a factor of 20. In water of the Ruhr ADBI was not detectable [Eschke et al., 1994a].
In an investigation into the presence of nitromusks and polycyclic musks in sewage sludge, AHTN and HHCB were the most important representatives of this group by far. AITI, ADBI and AHMI were also found at a level around 1 mg/kg, approximately a factor of 10 beneath the concentrations of AHTN and HHCB. Musk ketone was found three times (report limit 1 mg/kg), musk xylene was not detected in any sample (39 sludge samples) [Blok, 1997]. Based on the above mentioned data it may be assumed that the concentrations in water of the less used polycyclic musks will be lower than those of AHTN and HHCB by a factor 10 to 20 [Balk 1999].

Other fragrance ingredients

By RIZA acetyl cedrene was demonstrated in the Rhine and the Meuse in concentrations between 0.05 and 0.1 µg/l [Van Duyn, personal communication]. In the investigation by RITOX with the Empore disks besides AHTN and HHCB also limonene was demonstrated in the effluent of a sewage treatment plant [Van Loon et al. 1997].

6. BEHAVIOUR IN A SIMPLE PURIFICATION SYSTEM

All substances dealt with in this overview are organic and strongly hydrophobic. The logKows for the substances range from 4.3 to about 6 and the substances all are poorly soluble in water. In surface water the substances are therefore mainly bound to the organic fraction. If during the purification of drinking water use is made of a simple sand filter which also contains some biomass, it may be assumed that these substances will be trapped during passage and will be adsorbed onto the organic substance of the biomass.
Since these substances, except for limonene, are not readily degradable, adsorption onto sludge during the treatment process in a waste water purification plant is the main removal process as well. For the nitromusks and polycyclic musks adsorption onto sewage sludge has indeed been demonstrated [Blok, 1997].
Apart from adsorption, limonene will also biodegrade [SRC, 1996].

7. RISKS TO MAN AND THE ENVIRONMENT

7.1 Man

Almost every human will come into contact with fragrances. The contact can take place in various ways. The most direct and most common way is deliberate application onto the skin. Furthermore, contact is possible by way of remainders of fragrances which have stayed behind on washing (towels, pillows) [Groot and Frosch, 1997]. According to the same authors contact is also possible by way of physical contact with persons using fragrances. Inherent to the function of fragrances is the fact that they can be inhaled. Oral intake of fragrances from water or other drinks and food-stuffs also is a possible route [Groot and Frosch, 1997]. For HHCB an average odour threshold has been established of 5 μg/l (minimum 1.4 μg/l) whereas the substance can be tasted from 22 μg/l [Young et al., 1996]. These thresholds are higher than the concentrations occurring in surface water, so HHCB in samples of surface water will not be tasted or smelled.

Toxicity

Most reported effects concern allergies [Groot and Frosch, 1997]. In most tests on allergies AHTN and HHCB appeared to cause no reactions. The concentrations applied were at least a factor of 10 higher than the concentrations in consumer products. After carrying out irritation tests in accordance with Annex V of the EEC Directive 79/831, AHTN and HHCB were not classified as irritating. Investigations have demonstrated that these substances in man are not photo-irritating or photo-sensitising, in spite of some indications in that direction in test animals [Ford, 1998a].
Musk xylene (5%) is slightly irritating to man [RIFM, 1975] and may cause allergic reactions [Groot and Frosch, 1997]. These allergic reactions were not found in a test with 25 volunteers [RIFM Monograph, 1975]. Musk ketone is not irritating and does not cause allergic reactions to man [RIFM, 1975]. Limonene is irritating to the skin [Sax, 1987].

From subchronic oral studies in which rats were exposed to 1.5; 5; 15 and 50 mg/kg diet (AHTN) or 5; 15; 50 and 150 mg/kg diet (HHCB) during a period of 13 weeks No-Adverse-Effect Levels (NOEL) were calculated of 15 mg/kg$_{b.w.}$ for AHTN and 150 mg/kg$_{b.w.}$ for HHCB [Ford, 1998a]. On the basis of comparable studies with dermal exposure it may be assumed that the oral NOEL of AITI and ADBI are at the same level.

The NOELs for musk ketone and musk xylene, on the basis of teratogenicity studies, have been calculated as 7.5 mg/kg$_{b.w.}$/d, and 4.8 mg/kg$_{b.w.}$/d, respectively. The NOEL for musk ketone was derived from a multi-generation study in which female rats were exposed from the end of their gestation, through which the offspring was exposed by way of the milk. This offspring was monitored up to and including their offspring (F2). For musk xylene a similar test is still running. For both substances also a 90-day investigation has been carried out in which the effects on the development were followed after dermal exposure. The NOEL for musk xylene has been estimated from this by taking into account the dermal absorption [Ford, 1998b].
A very extensive toxicological investigation has been carried out into limonene (621 references in the RIFM database). In investigations with rats specific liver tumours appeared to occur. Therefore this substance was part of the National

Toxicology Programme in the early nineties. In the present opinion of EPA these tumours should be explained with a mechanism specific for the male rat. The lowest NOEL found is 100 mg/kg$_{b.w.}$/day. This NOEL has been established for dogs.

Exposure through direct contact

Establishing the systemic exposure to a substance, after a product has been applied to the skin, is difficult. Variables playing a part are: the preference of the consumer, the type of product ('leave-on' or 'wash-off'), the percentage of a substance in the product, the amount of product applied and the penetration of the substance through the skin. Ford [1998a] estimated the amounts of AHTN and HHCB which are applied on the skin per day through various products. For AHTN the quantity amounts to 18.36 mg/day and for HHCB 45.8 mg/day (both 'worst-case'). In studies into the penetration of a substance through the human skin a percentage of 1 to 2 was found. In a study with rats a penetration of 15% was found. If this latter value for penetration is used, it can be calculated that the daily systemic exposure for AHTN amounts to 2.8 mg/day (18.36 * 15%) and for HHCB 6.9 mg/day (45.8 * 15%). Corrected for a body weight of 70 kg a daily systemic exposure of 39 μg/kg$_{b.w.}$/day can be estimated for AHTN and 99 μg/kg$_{b.w.}$/day for HHCB ('worst case'). For other fragrances data for the estimation of the systemic exposure are absent.

Exposure through indirect contact

For this report exposure to fragrances in drinking water is, of course, a more relevant way than exposure by direct contact. Measurements of concentrations of fragrances in drinking water have not been found. Therefore, a 'worst case' scenario, i.e. the use of surface water as drinking water without any purification, was the basis for an indication of a possible contribution of drinking water to the pollution with fragrances. In chapter 4 it is indicated for various fragrances which concentrations are found in the Dutch surface water. The concentrations are given in table 4. For the estimation the concentration of a substance per litre is multiplied by the daily intake of drinking water. In the calculation an intake of drinking water of 2 litres/day and a body weight of 70 kg is assumed. The exposure is then converted to the amount of fragrance per kilogram body weight. The calculated exposure per day and per kilogram body weight is given in table 4.

Risk by direct exposure

The exposure is many times below the NOEL for AHTN and HHCB, so the risk is low. An extensive risk assessment will be carried out within the scope of the EC Directive for Cosmetics by the SCC (EC Committee on Cosmetics).
For this report the risk through indirect contact, the intake of drinking water, is more relevant.

Risk by indirect exposure

In table 4 the concentrations in surface water, the estimated exposure through drinking water and the lowest NOELs for a number of fragrances are given. For the exposure no allowance was made for the removal of substances during the purification of drinking water. The safety margin was calculated by comparing the exposure with the lowest NOEL of a substance. The safety margins have also been included in table 4. The safety margin which is very high for all fragrances, shows that the risk to man caused by the presence of fragrances in surface water is very low.

Table 4 Indication of the safety margins for man for the consumption of unpurified surface water as drinking water in connection with exposure to a number of fragrances.

	Concentration in surface water (µg/l) NL	elsewhere	Exposure of man through drinking water (µg/kg$_{l.g.}$/day)	Lowest NOEL (mg/kg$_{l.g.}$/day)	Safety margin
musk ketone	< 0.01 med.	0.03 Ruhr, meas.	<0.0003	7.5	$> 25 * 10^6$
musk xylene	< 0.01 med.	0.01 Ruhr, meas.	<0.0003	4.8	$> 16 * 10^6$
AHTN	0.11 90perc.	0.2 90perc.	0.003	15	$5 * 10^6$
HHCB	0.18 90perc.	0.5 90perc.	0.005	150	$30 * 10^6$
AITI	0.025^{e}		0.0007	$(15)^{**}$	$(21 * 10^6)$
AHMI	0.025^{e}		0.0007	$(15)^{**}$	$(21 * 10^6)$
ADBI	0.025^{e}		0.0007	$(15)^{**}$	$(21 * 10^6)$
Limonene	? (RITOX)			100	?
Acetyl cedrene	up to 0.05 - 0.1 (RIZA)		0.003	LD50 (oral) > 5000 mg/kg$_{l.g.}$	#

e estimated based on proportionality with AHTN, HHCB [Balk 1999]
** indication based on related structures
# If with acetyl cedrene, in connection with the short period of the toxicity test, a very high safety factor is applied (100,000), the safety margin is still greater than 1600.

7.2 Aquatic organisms

Toxicity

The aquatic toxicity has been investigated extensively for musk ketone, musk xylene, AHTN and HHCB. An overview of the data for the various substances is represented in table 5. Based on these data so-called PNECs (Predicted No Effect Concentration) have been calculated in accordance with the guidelines of the European Commission, the Technical Guidance Document for Environmental Risk Assessment of New and Existing Chemicals. To this end the lowest NOEC per substance was divided by an assessment factor of 10 or more. The PNEC for these substances varies between 0.001 and 0.007 mg/l.

For the other polycyclic musks no chronic toxicity data are available. Based on the structural relation with AHTN and HHCB, the PNEC for the other three polycyclic musks could be assumed to be 0.003 mg/l (level comparable to AHTN).
On the aquatic toxicity of acetyl cedrene no data were found. If the prediction of the toxicity is based on a so-called QSAR (quantitative structure activity relationships) for a mono-ketone, the toxicity is comparable to AHTN.
Recently a study showing extreme toxicity of one of the amino-derivatives of musk ketone [Behechti et al. 1998] could *not* be confirmed. The toxicity of all amino-derivatives of musk ketone are at the same level as the toxicity of parent substance [Putt 1999].

Table 5. Aquatic toxicity

	algae (mg/l)	*Daphnia magna* (mg/l)	Fish (mg/l)	PNEC (mg/l)
musk ketone [1]	72h-EC$_b$50 = 0.12 72h-NOEC = 0.09	21d-NOEC$_{repr}$ = 0.17	21d-NOEC = 0.063	0.0063
musk xylene [1]	5d-NOEC > 5.6	48h-EC50 > 5.6 21d-NOEC$_{repr}$ = 0.056	96h LC50 = 1.2 14d-NOEC$_{growth}$ < 0.1	0.0011
AHTN [2]	72h-NOEC$_b$ = 0.37 72h-EC$_b$50 = 0.47	21d-NOEC$_{repr}$ = 0.20	36d-NOEC$_{els}$ = 0.035	0.0035
HHCB [2]	72h-NOEC$_b$ = 0.20 72h-EC$_b$50 = 0.72	21d-NOEC$_{repr}$ = 0.11	36d-NOEC$_{els}$ = 0.068	0.0068
ADBI	-	-	-	**0.003
AITI [5]		48h-EC$_{50}$ = 0.42	-	**0.003
AHMI [5]	72h-NOEC = 0.044 ^{n} 72h-EC$_b$50 = 0.081 ^{n} -	48h-EC$_{50}$ = 0.32	96h-LC$_{50}$ = > 1.13 ^{n} 96h-NOEC = 0.9 ^{n}	**0.003
Acetyl cedrene	-	QSAR: 16d-EC50 = 0.04	-	-
Limonene	-	48h-EC50 = 69.6 [3@] (*D. pulex*) < 1 mg/l [4#]	< 1 mg/l [4#]	*** 0.0007

Source: [1] Tas et al. 1997, [2] Balk and Ford 1999b, [3] Passino and Smith 1987, [4] Haugaard 1995, [5] Balk 1999.
@ this value is above the estimated solubility in water in table 3
# acute toxicity to fish and daphnia both < 1 mg/l, lowest value 0.7 mg/l
** indication based on related structures with chronic toxicity data
*** indicative PNEC: lowest EC50: 1000

Exposure

The exposure of aquatic organisms equals the concentration measured in surface water. In order to take the variations in the concentrations in surface water in the course of time into account, the 90-percentile value of the measurements was as the exposure concentration (see also table 4).

Risks to aquatic organisms

In a risk assessment the exposure (PEC: predicted environmental concentration) is compared with a PNEC. If measured concentrations are available, those are used rather than the predicted concentrations. If the ratio PEC/PNEC ≥ 1, the concentration in the environment is exceeding the concentration where no effect on the ecosystem is expected. If values are ≤ 1 no direct effects are expected.
In table 6 the concentrations in Dutch surface water are compared with the PNEC values and the indications for PNEC values as represented in table 5. This shows that the distance between the concentrations measured in the water and the PNEC is very large. The ratio PEC/PNEC for AHTN and HHCB is 0.03 and 0.05, and for the other musks < 0.01. For limonene and acetyl cedrene the risk quotient cannot be estimated, but it is expected that the ratio is certainly not higher than that for the other fragrances.

Therefore, it may be concluded that the risk of exposure to these fragrances of aquatic organisms is very low.

Table 6. Risks to aquatic organisms

	Concentration in surface water (µg/l)		PNEC (µg/l)	Risk quotient NL $C_{surf.w.}$ / PNEC
	NL	elsewhere		
musk ketone	< 0.01 meas.	0.03 meas.Ruhr	6.3	<0.01
musk xylene	< 0.01 meas.	0.01 meas.Ruhr	1.1	< 0.01
AHTN	0.11 90perc.	0.2 90perc.	3.5	0.03
HHCB	0.18 90perc.	0.5 90perc.	6.8	0.05
AITI	0.025^{e}		** 3	< 0.01
AHMI	0.025^{e}		** 3	< 0.01
ADBI	0.025^{e}		**3	< 0.01
limonene	?		***0.7	?
acetyl-cedrene	up to 0.05-0.1		-	?

e estimated based on proportionality with AHTN, HHCB [Balk 1999]
** indication based on related structures
*** see table 5

8. POLICY

As a result of the risk assessments by RIVM, the Minister of VROM has concluded that there is no reason for concern or to take measures. The Minister will continue to follow the developments from the viewpoint of her policy. Industry expects that the consumption of both nitromusks and polycyclic musks will stabilise at a lower level. The Minister of VROM has requested the producers to report the use volumes of AHTN and HHCB in Europe on a voluntary basis. From this it should appear to what extent the use of these substances is being influenced by market effects[4].

Within the scope of the Existing Substance Regulation in the EC musk ketone and musk xylene have been put on the so-called third list of priority substances and the risks to man and environment will once more be evaluated according to the EC procedures. This will again be carried out under the responsibility of the Dutch authorities (RIVM/CSR). The results are not yet available.

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[4] The Consumers' Association has already reported on the replacement of nitromusks and polycyclic musks in cosmetic products by other fragrance ingredients [Consumentengids, 1997]

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