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TNO-report

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Phthalates and Artificial Musks in Perfumes

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Summary

Different groups of man-made chemicals are used as additives in consumer products. In perfumes these are especially phthalates and artificial musks. Because of the nature of the use of perfumes there is a high potential of human exposure. In addition these compounds will enter the environment during or after use of the products. For this reason 36 brands of perfumes are analysed to determine the content of phthalates and artificial musk compounds. Within the group of artificial musk compounds a distinction is made between nitromusks, polycyclic musks and macrocyclic musks.

The results show that phthalates and artificial musks are found in virtually every brand of perfume that was tested. The major phthalate was di-ethyl phthalate that was found in 35 of the 36 perfumes with concentration ranging from 0.4 to 22299 mg/kg and a median concentration of 1073 mg/kg. Other phthalates found are di-butyl phthalate, di-isobutyl phthalate and the well known di-2-ethylhexyl phthalate. To see whether low phthalate concentrations can be explained by the polymer tubing in the perfume bottle, this polymer was tested. The results suggest that the polymer is not the source of low levels of phthalates in the perfume.

Nitromusks were found in a limited number of samples only with one older type of perfume containing a musk ketone concentration of 4592 mg/kg. Polycyclic musks, and especially galaxolide (HHCB) and tonalide (AHTN) were found in all samples. The median concentration of HHCB was 73 mg/kg with a maximum concentration of 77848 mg/kg. AHTN is found almost as often as HHCB but in lower concentrations. Other polycyclic musks found in the perfumes are cashmeron (DPMI) and celestolide (ADBI).

29 samples are analysed to determine the presence of the newer macrocyclic musks. In total 6 of those artificial musks are identified in 21 of the 29 samples, with ethylene brassylate and exaltolide as the most prominent. Other macrocyclic musks found are muscone, civetone, ambrettolide and musconate. The finding that macrocyclic musks were found in 21 samples and that they appear to be the major musks in 11 of those samples seems to suggest that polycyclic musks are gradually replaced by macrocyclic musks.

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1. Introduction

1.1 Phthalates and artificial musks in perfumes

Nowadays, a large number of man-made chemicals are used as additives in consumer products[1,2]. As a result of their presence in these products the user is constantly exposed to these chemicals and they will enter the environment during or after use of the products[3]. That personal exposure leads to uptake of such chemicals in the human body is shown by the results of a number of studies into the presence of such compounds in human blood[4,5,6,7]. Meanwhile, numerous reports have shown the presence of such compounds in the environment. This report focuses on the use of phthalates and artificial musk compounds in perfumes.

Phthalates are a class of widely used industrial compounds that are generally applied as plasticizers in industrial products such as polyvinyl acetate, polyvinyl chloride (especially soft PVC), adhesives and coatings. As plasticizers they add flexibility to the synthetic organic polymers. Furthermore, these compounds are found in personal care products, especially in hair spray, fingernail polish and perfumes. They are ubiquitous in environmental samples due to their release during manufacture, use, and disposal of industrial and consumer products.

Artificial musk compounds are replacements for natural musk, an intensely smelling secretion of an abdominal gland of the male musk deer. They normally exhibit a strong, warm, sensual and long-lasting odour which makes them invaluable ingredients in perfumes, but also in fragrances for shampoos, fabric softeners, detergents, soaps and others. Artificial or synthetic musks are generally divided in three subgroups: nitro-musks, polycyclic musks and macrocyclic musks. As with the phthalates artificial musk compounds enter the environment due to release during manufacture, use, and disposal of consumer products.

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1 Peters RJB. Hazardous Chemicals in Consumer Products. TNO report R 2003/370, September **2003**.

2 Peters RJB. The Determination of Selected Additives in Consumer Products. TNO report R 2004/002, December **2003**.

3 Santillo D, Labunska I, Davidson H, Johnston P, Strutt M and Knowles O. Consuming Chemicals, Greenpeace Research Laboratories Technical Note 01/2003 (GRL-TN-01-2003), **2003**.

4 Peters RJB, Man-made Chemicals in Human Blood. TNO report R 2004/493, November **2004**.

5 CDC report: National Report on Human Exposure to Environmental Chemicals. CDC, Atlanta, Georgia, January **2003**.

6 WWF-UK National Biomonitoring Survey, November **2003**.

7 WWF Detox Campaign: Chemical Check Up: An analysis of chemicals in the blood of Members of the European Parliament, April **2004**.

2. Study objective and samples

2.1 Objective of the study

The objective of this study is to determine the presence of phthalates and artificial musk compounds, e.g. nitro-musks and polycyclic musks in perfumes. In addition it was decided to determine the presence of macrocyclic musks that are increasingly used as replacements for polycyclic musks. Table 1 gives an overview of the individual parameters that are determined in most of the samples.

Table 1 Overview of chemical parameters in this study.

Phthalates	Abbr.	Artificial musks	Abbr.
di-methyl phthalate	DMP	***nitromusks:***	
di-ethyl phthalate	DEP	musk ambrette	MA
di-iso-butyl phthalate	DIBP	musk ketone	MK
di-n-butyl phthalate	DBP	musk moskene	MM
benzylbutyl phthalate	BBP	musk tibetene	MT
di-cyclohexyl phthalate	DCHP	musk xylene	MX
di-(2-ethylhexyl) phthalate	DEHP		
di-n-octyl phthalate	DOP	***polycyclic musks:***	
di-iso-nonyl phthalate	DINP	galaxolide	HHCB
di-iso-decyl phthalate	DIDP	tonalide	AHTN
		traseolide	ATTI
		celestolide	ADBI
		cashmeran	DPMI
		phantolide	AHMI
		macrocyclic musks[A]:	
		ethylene brassylate	
		exaltolide	
		musk ambrettolide	
		civetone	

[A] Note that the results for these compounds will be only qualitative.

2.2 Samples

In total 36 samples are received from Greenpeace. All samples are common perfumes purchased by Greenpeace in common personal care product shops in The Netherlands. Table 2 provides an overview of all samples in this study.

Table 2 Overview of samples in this study.

TNO-code	Brand and name	Bar code number
52004338-01	Adidas: Floral Dream woman, eau de toilette, 50 ml	3 412244 310024
52004338-02	Etienne Aigner: Aigner in Leather man, eau de toilette, 30 ml	4 013670 312898
52004338-03	Armani: She eau de parfum, 50 ml	3 360372 061793
52004338-04	Blvgari: BLV notte man, eau de toilette, 50 ml	7 83320 88311
52004338-05	Hugo Boss: Boss in Motion eau de toilette, 40 ml	7 37052 85202
52004338-06	Calvin Klein: Eternity for Men eau de toilette, 50 ml	0 88300 60530
52004338-07	Coty: Celine Dion eau de toilette, 50 ml	3 412242 000033
52004338-08	Chanel: Chance eau de toilette, 50 ml	3 145891 264500
52004338-09	Yves Saint Laurent: Cinéma eau de parfum, 50 ml	3 365440 258914
52004338-10	FCUK: Him eau de toilette, 50 ml	8 70283 00201
52004338-11	Dior: Pure Poison eau de parfum, 50 ml	3 348900 606708
52004338-12	Gucci: Envy me eau de toilette, 50 ml	7 66124 01960
52004338-13	Isabella Rossellini: My Manifesto eau de parfum, 50 ml	3 412240 300104
52004338-14	Jean-Paul Gaultier: Le Mâle 75 ml	3 423470 317527
52004338-15	Joop!: Nightflight eau de toilette, 75 ml	3 414206 006914
52004338-16	Lancóme: Miracle, so Magic! 50 ml	3 147758 100754
52004338-17	Melvita: Iris Bleu eau de toilette, 100 ml	3 284410 008659
52004338-18	Naomi Campbell: Sunset eau de toilette, 50 ml	4 082800 500351
52004338-19	Mexx: Waterlove Man eau de toilette, 50 ml	4 082800 760106
52004338-20	Paco Rabanne: Excess pour Homme eau de toilette, 50 ml	3 349668 111541
52004338-21	Ralph Lauren: Polo Blue eau de toilette, 40 ml	3 360377 027619
52004338-22	Tommy Hilfiger: True Star 50 ml	0 22548 09746
52004338-23	Gloria Vanderbilt: Vanderbilt eau de toilette, 50 ml	3 357554 710013
52004338-24	Van Gils: Van Gils eau de toilette, 50 ml	8 710919 110029
52004338-25	Alqvimia: Aqua Natural 100 ml	8 420471 002723
52004338-26	Bogner: High Speed eau de toilette, 40 ml	7 66124 70041
52004338-27	The Body Shop: White Musk eau de perfume, 30 ml	5053 9130

TNO-code	Brand and name	Bar code number
52004338-28	Cartier: Le Baisier Du Dragon eau de parfum, 50 ml	3 432240 008435
52004338-29	Fiorucci: Fiorucci Loves you eau de toilette	8 002135 030688
52004288-01[A]	Puma: Jamaica eau de toilette, 30 ml	4 004711 801501
52004288-02[A]	Puma: Woman eau de toilette, 50 ml	4 004711 002267
52004280-04[B]	Calvin Klein: CK one for men, eau de toilette, 50 ml	0 88300 60768 6
52004280-05[B]	Jean-Paul Gaultier: Classique pour femmes, eau de toilette, 50 ml	3 423470 317152
52003227-06[C]	Dior: Poison eau de toilette, 50 ml	not determined
52003227-07[C]	Calvin Klein: Eternity for Women eau de toilette, 50 ml	not determined
52003227-08[C]	Chanel: No. 5 eau de parfum, 50 ml	not determined

[A] Samples of study 52004288 originally reported in TNO report TR 04/516, “The determination of phthalates and musks in two perfumes”, November 3, 2004.

[B] Samples of study 52004280 originally reported in TNO report R 2005/***, “Man-made Chemicals in Personal Care Products”, January 2005.

[C] Samples of study 52003227 originally reported in TNO report R 2003/370 “Hazardous Chemicals in Consumer Products”, September 2003.

3. Methods and materials

3.1 Sampling and sample pre-treatment

Samples are stored at room temperature until analysis. All samples were clear liquids and no special pre-treatment was applied apart from homogenisation by shaking for 1 minute before collecting a sub-sample for analysis.

3.2 Analytical procedures

A sub-sample of each sample was mixed with hexane/acetone and an internal standard was added. The mixture was shaken and heated in a water bath. Next, the vial was sonicated until a homogenous suspension resulted, and cooled in a refrigerator. A part of the clear liquid was collected and an injection standard was added. The analysis of phthalates and artificial musks is performed using gas chromatography in combination with mass spectrometry (GC/MS). The GC/MS is a Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column, length 30 m, 0.25 mm i.d., 0.25 μm film thickness, and interfaced to a Hewlett Packard 5973 mass spectrometer. For the specific determination of phthalates, nitro and polycyclic musks the mass spectrometer is used in the selected ion monitoring mode. For the GC/MS screening of macrocyclic musks the same instrument is used in the scan mode, allowing the identification of peaks in the chromatogram based on their mass spectrum.

3.3 Calculation of results

Identification of phthalates, nitro- and polycyclic musks is based on retention time and qualifier ion ratios. Quantification is based on external standards analysed within the same series as the sample extracts. The external standards are prepared from commercially available pure substances or standard solutions. In all cases peak areas are used for calculations.

The identification of macrocyclic musks in the GC/MS screening is based on a comparison of full scan mass spectra from the chromatographic peaks with reference mass spectra in a NIST/EPA/NIH Mass Spectral Library and the NBS75K library in the HP ChemStation. Only peaks with a minimum peak area, corresponding to a concentration of approximately 1000 mg/kg in the product are considered. Peaks are considered positively identified if the correlation between spectra and library spectra is better than 90%.

In general results of all analysis are expressed in mg/kg product. In summary tables for some compounds median concentrations and the 75- and 90-percentiles are

given. These are calculated only for compounds that are observed in at least 30% of the samples. When calculating such concentrations all measurement results (including those below the reporting limit) are included.

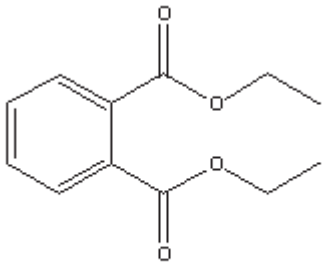
4. Results

4.1 Phthalates

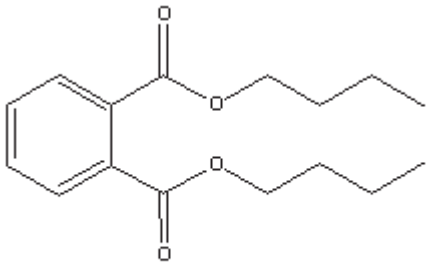
4.1.1 General information

Phthalates are commonly found in cosmetic and personal care products, especially in nail polish, hair sprays and perfumes. In perfumes phthalates are used as a carrier or solvent for artificial musks. The most important phthalate for this application is diethyl phthalate (DEP). The Swedish Society for Nature Conservation performed an investigation about how common phthalates are in cosmetic products[8]. Most products contained phthalates and in many cases DEP was the major phthalate with concentrations up to 19,000 mg/kg for perfumes. Dibutyl phthalate (DBP) was found also but to a much lesser extent.

Due to the presence of phthalates in common household products, cosmetics and toys, the potential for human exposure is very high. In 2001 the EU has imposed an emergency restriction for 6 phthalates (DBP, BBP, DEHP, DOP, DINP and DIDP) in articles for children in the age of 0-3 years[9]. Scientists at the US Centre for Disease Control have documented human exposure to phthalates by determinations of the mono-ester metabolites in human urine[5]. Interesting was that urinary levels of mono-ethyl phthalate (the metabolite of DEP) was found to be lower for ages 6-11 years than for the 12-19 years age group and the 20 years and older age group (trending in an opposite direction from other phthalates). In addition, levels in females were higher than levels in males. In a recent TNO study (conducted for Greenpeace) DEP was also found in human blood samples, although, less frequently and in lower concentrations than DBP and di-ethylhexyl phthalate (DEHP). The chemical structure of DEP and DBP is presented below.



di-ethyl phthalate



di-butyl phthalate

[8] Swedish Society of Nature Conservation. Phthalates in European Cosmetic Products. November, **2002**.

[9] Rastogi SC, Worsoe IM. Danish National Environmental Research Institute. NERI Technical Report No. 373, **2001**.

4.1.2 Results for phthalates in this study

All products are analysed for phthalates and 35 of the 36 samples did contain one or more phthalates. Only 1 sample, sample 4338-23, did not contain any of the phthalates and 3 more samples contained phthalates in (individual) concentrations below 10 mg/kg. As expected the highest phthalate concentrations are found for di-ethyl phthalate (DEP). The DEP concentrations ranged from 0.4 to 22299 mg/kg with a median concentration of 1073 mg/kg indicating that half of the perfumes contain DEP concentrations higher than this. DEHP another phthalate commonly found in many products is found in 19 perfumes ranging from 1.0 to 167 mg/kg. Other phthalates frequently encountered are DBP, DIBP and to a lesser extent DMP. Surprisingly, DMP was found in a high concentration in one of the samples only. A summary of the results is given in the table 3. The complete results of the phthalate analysis are presented in table 1 in the appendix.

Table 3 Summary results of phthalates in perfumes.

Summary phthalates	Reporting limit	Maximum concentration	50-perc. (median)	75-perc.	90-perc.	N out of 36
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	
DMP	0.1	2982		0.7	1.7	16
DEP	1	22299	1187	4009	6954	34
DIBP	0.1	38	0.5	4.1	5.6	20
DBP	0.1	14	0.2	1.2	3.0	21
BBP	0.1	110				9
DCHP	0.1	3				1
DEHP	1	167		2.9	23	14
DOP	0.1	0				0
DINP	1	26				1
DIDP	1	37				5

4.1.3 The origin of low concentrations of phthalates

When high concentrations of DEP are found it is clear that they are intentional additions to the product. This is different when low concentrations of DEP are found. These may still have been added, may be present due to impurities in one of the base materials of the perfume, or may originate from the polymer parts of the spraying system, especially the polymer tubing inside the bottle since this is permanently in contact with the perfume.

To determine the origin of DEP in a perfume containing low concentrations of DEP (sample 4288-01 and -02), the polymer parts of the sample were sampled and leaching of DEP from this material was determined using a 2% dichloromethane solution in ethanol. In addition the polymer itself was extracted and the type of polymer was determined. The results show that the tubing inside both bottles is

made of polypropylene, a polymer that to our knowledge normally does not contain phthalates.

The analysis of the leaching fluids and the polymer itself show that the phthalates are the same as those found in the perfume itself. Even the ratios between the amounts of individual phthalates in the eau de toilette, the leaching fluid and the polymer are comparable (see table 4) indicating that they all have a common source. Please note that the fact that the concentrations are also similar is only co-incidental since the concentrations in the perfume, the leaching fluid and the polymer have different units.

The absolute amounts of phthalates found in the leaching fluid and in the polymer itself are low compared to the amounts found in the original perfume. From the concentration of DEP in the perfume, the amount of perfume in the bottle, and the weight of the polymer tubing in the perfume, one can easily calculate that the DEP concentration in the polymer tubing should have been in the order of 1000 mg/kg to explain the DEP concentration found in the perfume. These findings suggest that the phthalates migrated from the perfume into the polymer tubing and not the other way around.

Table 4 Phthalates in perfume sample 52004288-01 and -02 and in the polymer parts of the perfume bottles.

Sample part	Eau de toilette		Leaching fluid		Polymer tubing	
TNO code	4288-01	4288-02	4288-01	4288-02	4288-01	4288-02
unit	mg/kg	mg/kg	ng/ml	ng/ml	mg/kg	mg/kg
DMP	<	<	<	<	<	<
DEP	37	27	43	26	0.97	0.73
DIBP	5.5	1.9	2.9	3.0	0.04	0.09
DBP	2.9	1.8	2.1	1.2	<	<
BBP	<	<	<	<	<	<
DCHP	<	<	<	<	<	<
DEHP	25	<	31	<	0.79	<
DOP	<	<	<	<	<	<
DINP	<	<	<	<	<	<
DIDP	<	<	<	<	<	<

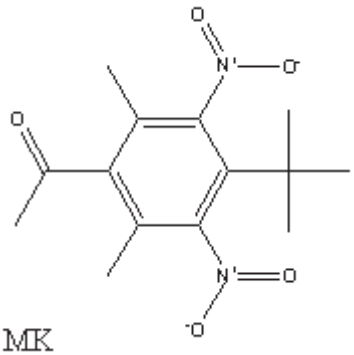
4.2 Artificial musks

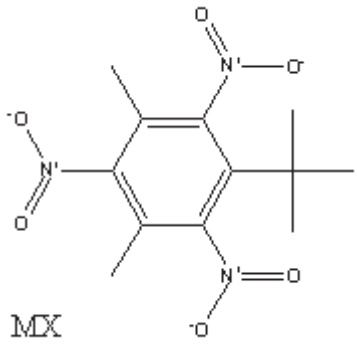
4.2.1 General information

Natural Musk is an intensely smelling secretion of an abdominal gland of the male musk deer serving the purpose of a male sexual scent signal. Since ancient times it is aspired to humans who have used it for medicines and as a fixative in perfumes.

However, the increasing demand almost resulted in the extinction of the musk deer, and the production of artificial musk fragrances. Artificial musk fragrances are generally divided in three subgroups: nitromusks, polycyclic musks and macrocyclic musks.

Nitromusks have accidentally been discovered in 1888[10] by Baur. He later found the better known musk xylene (MX), musk ketone (MK) and musk Ambrette (MA), all of them nitration products of aromatic compounds. Musk Tibetene (MT) and Moskene (MM) are also members of this group. Due to its photosensitivity[11] and neurotoxic effects[12] MA is not used any more and MK and MX are the most common of the nitromusks in perfumes. Due to the toxicity and persistence of nitromusks, the production and use of MA is prohibited while MX and MK should be phased out in 2010[13]. In a study by the Dutch Keuringsdienst van Waren in 1997, 114 cosmetic products were tested for nitromusks[14]. MK was the most prominent musk and found in about 50% of the samples, with concentrations ranging from 1 to 24000 mg/kg, the latter found in perfume. In a more recent TNO study MK was found in a single product only, indicating the diminishing use of nitromusks[1]. The chemical structures of MK and MX are shown below.





Polycyclic musks have been developed in the middle of the twentieth century and have gradually replaced the nitromusks during the last ten years. The best known and most used polycyclic musks are tonalide (AHTN) and especially galaxolide (HBCD). Due to their extensive use polycyclic musks have been found in the environment[15,16], in human fat, milk[17] and blood[4]. Although polycyclic musks have

10 Baur A. Ber. Dtsch. Chem. Ges. 24, 2832, **1891**.

11 Cronin E. Contact Dermatitis 11, 88, **1984**.

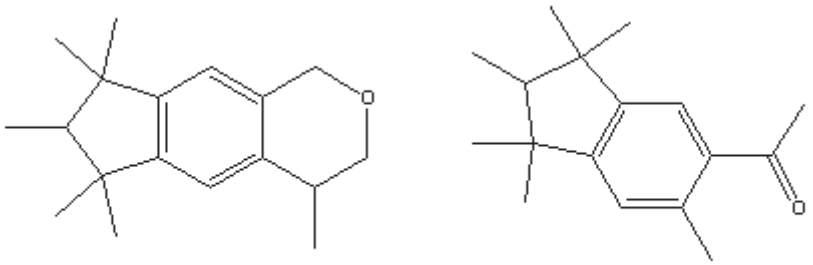
12 Spencer PS, Bischoff-Fenton MC, Moreno OM, Opdyke DL, Ford RA, Toxicol. Appl. Pharmacol. 75, 571, **1984**.

13 Bester K, Hühnerfuss H, Lange W, Rinkus GG, Theobald N. Water Res. 32, 1857-1858, **1998**.

14 Rooselaar J, Weijland JW. De bepaling van nitromuskverbindingen in kosmetische producten met behulp van GC en GC/MS. Project EN 94-3, February **1997**.

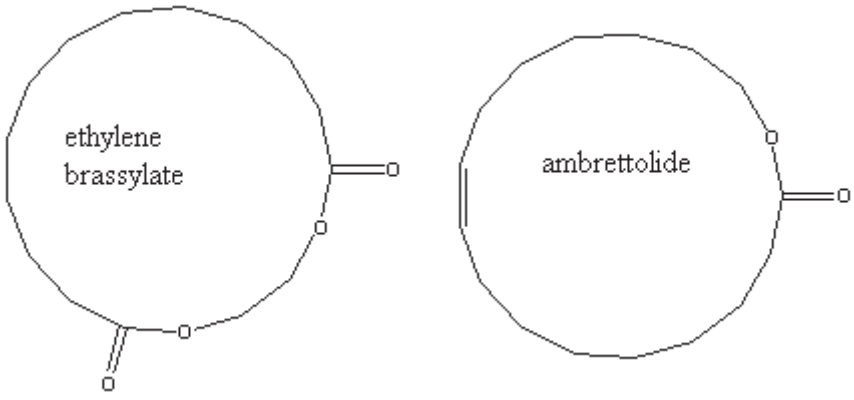
15 Eshke HD. Z. Umweltchem Oekotox 6, 183-189, **1994**.

been tested in the past and showed no toxicological and dermatological effects, their high levels of use, chemical stability and low biodegradability make polycyclic (and nitromusks) potential environmental contaminants due to their bioaccumulation. As a consequence polycyclic musks are gradually replaced by another group of musks, the macrocyclic musks. The chemical structures of HHCB and phantolide are shown below.



HBCD phantolide

The group of macrocyclic musks consists of partially artificial and partially nature-identical members. Macrocyclic chemistry started with the structural elucidation of Muscone, the macrocyclic component of the secretion of the musk deer in 1926. However, macrocyclic musks are not only found in animals but also in plants. An example is Exaltolide that is found in angelica root oil. Together with Ethylene brassylate (trade name Musk T) it is the most used macrocyclic musk. Others are Ambrettolide and Civetone. The chemical structure of macrocyclic musks suggests an easy microbial decomposition which, however, has yet to be confirmed. The chemical structures of Ethylene brassylate and Ambrettolide are shown below.



[16] Peters RJB. Hazardous Chemicals in Precipitation, TNO report R 2003/198, May **2003**.

[17] Rimkus GG. Chemosphere 33, 2033-2043, **1996**.

4.2.2 Results for artificial musks in this study

Nitro- or polycyclic musks are found in all 36 samples. In 2 samples the concentrations of the individual musks are below 1 mg/kg and in 9 samples they are below 10 mg/kg. As expected HHCB is the most common polycyclic musk and is found in all samples in concentrations ranging from 0.1 to 77848 mg/kg. The median concentration is 73 mg/kg indicating that in half of the perfumes the concentration is higher than 73 mg/kg. The 75-percentile indicates that in about 9 samples the concentrations of HHCB are above 17000 mg/kg.

Another polycyclic musk, AHTN, is found in 34 of the 36 samples. The AHTN concentrations range from 0.1 to 26200 mg/kg with a median concentration of 18 mg/kg. Different from HHCB, the 75-percentile for AHTN is only 111 mg/kg, indicating that while AHTN is commonly found, there are only a limited number of samples that contain high AHTN concentrations. ADBI and DPMI are two other polycyclic musks that were found in about half of the samples while AHMI and ATTI were found in about a quarter of the samples.

Nitromusks were only found in a limited number of samples. The most frequently found are MX and MK in about 20% of the samples. With the exception of MK, that was found in one sample in a concentration of 4592 mg/kg, the concentrations of the nitromusks were low when compared to the polycyclic musks. As observed in other studies, the nitromusks seem to be mostly replaced by the polycyclic musks. A summary of the results for the nitro- and polycyclic musks is given in table 5. The full results are in table 2 in the appendix.

Table 5 Summary results of artificial nitro- and polycyclic musks in perfumes.

Summary nitro- and polycyclic musks	Reporting limit	Maximum concentration	50-perc. (median)	75-perc.	90-perc.	N out of 36
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	
ADBI	0.1	133	0.1	10	23	17
AHMI	0.1	42		3.0	11	11
AHTN	0.1	26200	19	139	7550	33
ATTI	0.1	512				7
DPMI	0.1	698		150	263	16
HHCB	0.1	77848	73	17540	25990	36
MA	0.1	0				0
MK	0.1	4592				7
MM	0.1	15				1
MT	0.1	1				6
MX	0.1	15				8

Because of their potential for bioaccumulation polycyclic musks gradually seem to be replaced by macrocyclic musks. Based on their structure the latter may be more readily biodegradable than the polycyclic musks. To get an impression of the current use of macrocyclic musks, a GC/MS screening was applied on the first 29 samples to determine their presence in the perfumes. It should be kept in mind that the results for these compounds are only qualitative. The identification limit was conservatively set at 1000 mg/kg.

In total 6 different macrocyclic musks are identified in 21 of the 29 samples investigated. These are Muscone, Civetone, Ambrettolide, Exaltolide, Ethylene brassylate and Musconate. The most frequently found macrocyclic musk is Ethylene brassylate that was found in 15 of the 29 samples. Based on the peak areas in the chromatograms the concentrations of Ethylene brassylate are expected to be at least of the same order as those of HHCB and AHTN. To a lesser extent this is also the case for Exaltolide that is found in about one third of the samples. One of the reasons for the high additions may be that the odour profiles (intensity, odour threshold etc.) of macrocyclic musks are different from those of the nitro- and polycyclic musks and simply more is needed to achieve a similar profile. The macrocyclic musks and the number of times they were identified in the samples are given in table 6. The more detailed qualitative results are in table 3 in the appendix.

Table 6 Summary results of artificial macrocyclic musks in perfumes (only qualitative).

Summary macrocyclic musks	Identification limit	maximum concentration	50-perc. (median)	75-perc.	90-perc.	N out of 29
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	
muscone	1000					1
civetone	1000					2
ambrettolide	1000					6
exaltolide	1000					11
ethylene brassylate	1000					15
musconate	1000					1

4.2.3 Are macrocyclic musks replacing polycyclic musks?

Macrocyclic musks were found in 21 of the 29 samples to which the GC/MS screening was applied. In 11 of these samples the macrocyclic musks appear to be the major musks present in the sample. In only 4 of these 29 samples polycyclic musks are the major musks. Then, there are 9 products that contain high concentrations of both, macro- and polycyclic musks, while 5 products contain lower amounts of musks (that is the sum of the nitro- and polycyclic musks is below 100 mg/kg and no macrocyclic musks are identified). These results clearly indicate that in perfumes macrocyclic musks are gradually replacing the older polycyclic musks, like the latter have replaced the nitromusks. The fact that there are also products

that contain both, macro- and polycyclic musks may indicate that this replacement is not always straightforward.

4.3 Quality control measurements

4.3.1 Method validation parameters

All methods applied were already used on earlier occasions and were validated according to research and development protocols. The linearity of the instrumental analysis is determined but the linearity of the complete method is not a very useful parameter since the concentrations in the products can be very far apart. In general, extracts exceeding the linear range are diluted for the result to fall in the linear range of the instrumental analysis.

The repeatability and recoveries for each of the methods is determined by replicate analyses (n = 6) of one sample and one sample spiked with the complete set of analytes. For phthalates the average repeatability in this matrix is 5% with a maximum of 10% for DINP and DIDP. The average recovery was 95%. For the nitro- and polycyclic musks the average repeatability is 6% with a maximum of 8% for HHCB with an average recovery of 99%.

The quantification limits for the phthalates, nitro- and polycyclic musks are given in the result tables in the appendix and generally vary between 0.1 mg/kg and 1 mg/kg, depending on the specific chemical parameter and the blank value. For the macrocyclic musks the identification limit is set at 1000 mg/kg.

4.3.2 Recovery of extraction standard

To all samples an extraction standard (di-phenyl phthalate) was added. The recovery of this extraction standard was above 85% in all cases. No correction is applied for this recovery.

4.3.3 Blank samples

With each series blank samples were included. These blank consisted of a complete analysis in the same series as the samples, however, without the addition of sample material. With the exception of the phthalates DEP and DEHP no blank values were observed. The blank values observed for DEP and DEHP were 0.2 mg/kg and 0.5 mg/kg. Results were corrected for this blank value and the reporting limit for these phthalates was raised to 1 mg/kg.

5. Conclusions

In this study 36 perfumes have been tested for the presence of phthalates and artificial musks. The following conclusions are drawn from the results:

- Phthalates are found in 35 of the 36 samples. One perfume did not contain any phthalate. As expected for perfumes DEP was the phthalate found most frequently and in the highest concentrations. DEP was found in a median concentration of 1073 mg/kg and a maximum concentration of 22299 mg/kg. Other phthalates were DBP, DIBP and DEHP, mostly in concentrations below 10 mg/kg.
- The analysis of the polymer parts of one sample bottle suggest that it is unlikely that low concentrations of phthalates in the perfume originate from the polymer tubing inside the bottle
- Nitromusks are found only in a limited number of perfumes with MK and MX as the most prominent members. One older type of perfume contained a relatively high concentration of MK, 4592 mg/kg. It seems that nitromusks by now are mostly replaced by other artificial musks.
- Polycyclic musks are found in all samples. By far the most prominent is HHCB that was found in all samples, in a median concentration of 73 mg/kg and a maximum concentration of 77848 mg/kg. AHTN is found almost as often as HHCB but in lower concentrations. DPMI and ADBI are found in about half of the samples.
- Macrocyclic musks are identified in 21 of the 29 perfumes tested. The 6 macrocyclic musks that are identified are Muscone, Civetone, Ambrettolide, Exaltolide, Ethylene brassylate and Musconate. Ethylene brassylate and Exaltolide are the major macrocyclic musks found in respectively 15 and 11 of the 29 samples.
- From the results it seems that macrocyclic musks are replacing polycyclic musks since there are 11 perfumes containing mainly macrocyclic musks and 4 perfumes containing mainly polycyclic musks. There are, however, still 9 perfumes that contain both, and in high concentrations. 5 perfumes contain only limited amounts of these artificial musks.

6. QA/QC statement

The analytical determinations in this study are performed in compliance with NEN-EN-ISO/IEC 17025 and STERLAB accreditation no. 54, “The development and application of methods for the determination of organic contaminants in environmental matrices, wastes and materials”. TNO Environment, Energy and Process Innovation is listed in the STERLAB register under no. L 026. STERLAB is part of the Dutch Council for Accreditation (RvA) and is a member of the European co-operation for Accreditation (EA) and the International Laboratory Accreditation Co-operation (ILAC). TNO Environment, Energy and Process Innovation operates in compliance with the Quality System standard ISO 9001 (certificate no. 07246-2003-AQ-ROT-RvA).

7. Authentication

Name and address of the principal:
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Name and functions of the cooperators:

Dr. R.J.B. Peters	Project Leader
Ing. H. Beeltje	Technician

Names and establishments to which part of the research was put out to contract:
-

Date upon which, or period in which, the research took place:
December 2004 – January 2005

Signature:

R.J.B. Peters
Project leader MA

Approved by:

Dr. M.P. Keuken
Head of Department MA

Appendix Full results of all product analysis

When reading the tables in this appendix please note that while results are always rounded to the correct decimal number, they are not always rounded to the correct number of significant units. Due to the uncertainty in the results (for instance the repeatability of the method), the number of significant units is limited. This is especially true for concentrations of several thousands of mg/kg are reported. In general no more than two significant numbers apply, so that for DEP in sample 52004338-17 (table 1) the result of 11189 mg/kg should be read as 11000 mg/kg.

Please note that in the results tables the reporting limits for samples with TNO code 4280-04 and -05 and 3227-06 to -08 for phthalates as well as for the artificial musks are different from the 31 other samples. This is clearly indicated in the tables. Results for macrocyclic musks are only available for samples 4388-01 to -29.

While the names for the phthalates are well known and straightforward, those for the artificial musks are not and several trade names are used in the literature. Therefore, the CAS numbers, official names and some common trade names of these musks are listed below.

Nitromusks:

- MA: 2,6-dinitro-3-methoxy-4-t-butyl-toluene: CAS 83-66-9: Musk Ambrette
- MK: 4,6-dinitro-2-acetyl-5-t-butyl-toluene: CAS 81-14-1: Musk Ketone
- MM: 4,6-dinitro-1,1,3,3,5-pentamethyl-indane: CAS 116-66-5: Musk Moskene
- MT: 2,6-dinitro-3,4,5-trimethyl-1-t-butyl-benzene: CAS 145-39-1: Musk Tibetene
- MM: 2,4,6-trinitro-5-t-butyl-xylene: CAS 81-15-2: Musk Xylene

Polycyclic musks:

- DPMI: 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone: CAS 1922-67-4: Cashmeron.
- ADBI: 4-acetyl-1,1-dimethyl-6-t-butyldihydro-indene: CAS 13171-00-1: Celestolide, Crysolide.
- HHCB: 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-2-benzopyran: CAS 1222-05-5: Galaxolide, Musk GX, Abbalide, Musk 50, Pearlide.
- AHMI: 5-acetyl-1,1,2,3,3,6-hexamethyl-indane: CAS 15323-35-0: Phantolide .
- AHTN: 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene: CAS 1506-02-1: Tonalide, Fixolide, Tetralide.
- ATTI: 5-acetyl-1,1,2,6-tetramethyl-3-isopropyl-indane: CAS 68140-48-7: Traseolide.

Macrocyclic musks:

- Muscone: 3-methyl-cyclopentadecanone: CAS 541-91-3.
- Exaltolide: oxacyclohexadecan-2-one: CAS 106-02-5: Cyclopentadecanolide, Pentalide, Thibetolide.
- Ambrettolide: Z-oxacyclo-heptadec-8-en-2-one: CAS 123-69-3.
- Ethylene brassylate: 1,4-dioxacycloheptadecane-5,17-dione: CAS 105-95-3: Astratone, Musk T.
- Civetone: Z-9-cycloheptadecen-1-one: CAS 542-46-1.
- Musconate: 1,4-dioxacyclo-hexadecane-5,16-dione: CAS 54982-83-1.

Table 1 Phthalates in 36 samples of perfume.

sample code TNO		4338-01	4338-02	4338-03	4338-04	4338-05	4338-06	4338-07	4338-08	4338-09	4338-10
sample code Greenpeace		1	2	3	4	5	6	7	8	9	10
sample description	reporting limit	Adidas, Floral Dream	Aihner, In Leather	Armani, Emporio	Bulgari, BLV Notte	Boss, Hugo Boss	Calvin Klein, Eternity	Celine Dion	Chanel, Chance	Cinéma, Yves Saint Laurent	FCUK, Him
unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
DMP	0.1	0.3	0.8	1.3	<	1.9	<	1.7	<	0.7	0.1
DEP	1	1301	1909	1383	3902	2.3	8232	4072	19	102	4.8
DIBP	0.1	5.8	3.8	3.0	3.1	1.7	2.9	3.5	<	<	<
DBP	0.1	<	0.7	0.8	<	0.1	0.9	3.1	2.1	<	1.1
BBP	0.1	<	<	<	<	<	<	<	0.9	<	<
DCHP	0.1	<	<	<	2.9	<	<	<	<	<	<
DEHP	1	<	12	<	<	<	1.2	<	<	<	<
DOP	0.1	<	<	<	<	<	<	<	<	<	<
DINP	1	<	<	<	<	<	<	<	<	<	<
DIDP	1	<	<	<	<	<	<	10	<	<	1.5

sample code TNO		4338-11	4338-12	4338-13	4338-14	4338-15	4338-16	4338-17	4338-18	4338-19	4338-20
sample code Greenpeace		11	12	13	14	15	16	17	18	19	20
sample description	reporting limit	Dior, Pure Poison	Gucci, Envy Me	Isabella, Rossellini, My Manifesto	Jean Paul Gaultier, Le Male	Joop, Nightflight	Lancóme, Miracle So Magic!	Melvita, Iris Blue	Naomi Campbell, Sunset	Mexx, Waterlove	Paco Rabanne, Excess pour homme
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
DMP	0.1	<	<	0.6	0.4	<	<	0.1	1.1	<	0.3
DEP	1	29	25	1553	9884	3988	0.4	11189	1.2	18	2822
DIBP	0.1	3.9	4.9	8.7	<	0.2	5.2	<	<	<	4.9
DBP	0.1	2.5	<	<	<	<	<	0.7	<	0.4	0.2
BBP	0.1	<	<	<	1.0	<	<	77	0.1	<	<
DCHP	0.1	<	<	<	<	<	<	<	<	<	<
DEHP	1	<	2.3	<	<	1.7	<	4.9	<	6.0	7.5
DOP	0.1	<	<	<	<	<	<	<	<	<	<
DINP	1	<	<	<	<	<	<	<	<	<	<
DIDP	1	<	<	<	<	<	<	<	2.1	11	<

Table 1 (continued). Phthalates in 36 samples of perfume.

sample code TNO		4338-21	4338-22	4338-23	4338-24	4338-25	4338-26	4338-27	4338-28	4338-29
sample code Greenpeace		21	22	23	24	25	26	27	28	29
sample description	reporting limit	Polo, Ralph Lauren Blue	Tommy Hilfiger, True Star	Vanderbilt, Gloria Vanderbilt	Van Gils, Eau De Toilette	Aqua Natural	Bogner, High Speed	The Body Shop, White Musk, Eau De Parfume	Cartier, Le Baiser Du Dragon	Fiorucci Loves You
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
DMP	0.1	1.2	1.9	<	<	1.7	<	2982	<	<
DEP	1	5338	225	<	5637	1667	<	37	4533	2190
DIBP	0.1	<	<	<	5.3	0.8	<	<	<	0.2
DBP	0.1	0.2	0.2	<	1.5	6.0	<	<	<	0.2
BBP	0.1	<	<	<	<	110	0.1	0.6	0.3	0.3
DCHP	0.1	<	<	<	<	<	<	<	<	<
DEHP	1	<	<	<	1.1	<	<	<	<	<
DOP	0.1	<	<	<	<	<	<	<	<	<
DINP	1	<	<	<	<	<	<	<	26	<
DIDP	1	<	<	<	<	<	37	<	<	<

sample code TNO		4288-01	4288-02	4280-04	4280-05	3227-06	3227-07	3227-08
sample code Greenpeace		30	31	32	33	35	36	37
sample description	reporting limit	Puma, Jamaica man	Puma, woman	CK one, for men, Eau De Toilette	Jean Paul Gaultier, Classique	Christian Dior, Poison	Calvin Klein, Eternity woman	Chanel No. 5
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
DMP	0.1	<	<	< 1	< 1	< 1	< 1	< 1
DEP	1	37	27	1073	785	5675	22299	325
DIBP	0.1	5.5	1.9	< 1	< 1	33	38	< 1
DBP	0.1	2.9	1.8	< 1	1	14	14	< 1
BBP	0.1	<	<	< 1	< 1	< 1	< 1	< 1
DCHP	0.1	<	<	< 1	< 1	< 1	< 1	< 1
DEHP	1	25	<	76	1	167	88	20
DOP	0.1	<	<	< 1	< 1	< 1	< 1	< 1
DINP	1	<	<	< 1	< 1	< 1	< 1	< 1
DIDP	1	<	<	< 1	< 1	< 1	< 1	< 1

Table 2 Nitro- and polycyclic musks in 36 samples of perfume.

sample code TNO		4338-01	4338-02	4338-03	4338-04	4338-05	4338-06	4338-07	4338-08	4338-09	4338-10
sample code Greenpeace		1	2	3	4	5	6	7	8	9	10
sample description	reporting limit	Adidas, Floral Dream	Aihner, In Leather	Armani, Emporio	Bulgari, BLV Notte	Boss, Hugo Boss	Calvin Klein, Eternity	Celine Dion	Chanel, Chance	Cinéma, Yves Saint Laurent	FCUK, Him
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
ADBI	0.1	<	<	2.4	20	<	9.2	7.9	<	8.3	17
AHMI	0.1	<	<	<	3.1	<	11	1.1	<	2.0	2.8
AHTN	0.1	18	32	53	1751	1.3	7273	111	17	88	73
ATTI	0.1	<	0.1	<	<	<	<	<	<	<	<
DPMI	0.1	3.3	232	3.6	698	271	<	164	<	<	278
HHCB	0.1	73	20	8972	26350	7.2	19970	18463	18	17232	19476
MA	0.1	<	<	<	<	<	<	<	<	<	<
MK	0.1	<	0.5	<	<	<	<	<	<	0.2	<
MM	0.1	<	<	<	<	<	<	<	<	<	<
MT	0.1	0.7	<	<	<	<	<	1.1	<	<	<
MX	0.1	<	<	<	<	0.1	<	<	<	<	<

sample code TNO		4338-11	4338-12	4338-13	4338-14	4338-15	4338-16	4338-17	4338-18	4338-19	4338-20
sample code Greenpeace		11	12	13	14	15	16	17	18	19	20
sample description	reporting limit	Dior, Pure Poison	Gucci, Envy Me	Isabella, Rossellini, My Manifesto	Jean Paul Gaultier, Le Male	Joop, Nightflight	Lancóme, Miracle So Magic!	Melvita, Iris Blue	Naomi Campbell, Sunset	Mexx, Waterlove	Paco Rabanne, Excess pour homme
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
ADBI	0.1	0.2	<	<	30	<	<	<	0.1	<	8.3
AHMI	0.1	<	<	<	42	<	<	<	<	<	15
AHTN	0.1	<	<	2.8	26200	1.2	0.7	0.7	0.4	0.3	8507
ATTI	0.1	<	<	<	512	0.3	<	<	<	0.5	1.7
DPMI	0.1	<	192	2.0	<	<	<	<	<	150	170
HHCB	0.1	1.4	0.4	9.0	37644	8.8	2.0	44	1.3	0.5	0.8
MA	0.1	<	<	<	<	<	<	<	<	<	<
MK	0.1	<	<	<	<	<	<	<	<	<	11
MM	0.1	<	<	<	<	<	<	<	<	<	15
MT	0.1	<	<	0.3	<	<	0.3	<	<	0.4	<
MX	0.1	0.4	<	<	<	0.2	<	<	<	0.1	15

Table 2 (continued). Nitro- and polycyclic musks in 36 samples of perfumes.

sample code TNO		4338-21	4338-22	4338-23	4338-24	4338-25	4338-26	4338-27	4338-28	4338-29
sample code Greenpeace		21	22	23	24	25	26	27	28	29
sample description	reporting limit	Polo, Ralph Lauren Blue	Tommy Hilfiger, True Star	Vanderbilt, Gloria Vanderbilt	Van Gils, Eau De Toilette	Aqua Natural	Bogner, High Speed	The Body Shop, White Musk, Eau De Parfume	Cartier, Le Baiser Du Dragon	Fiorucci Loves You
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
ADBI	0.1	5.5	23	<	17	<	<	133	50	<
AHMI	0.1	9.2	3.7	<	7.4	<	<	28	<	<
AHTN	0.1	7827	110	0.1	383	0.1	0.3	16060	222	0.9
ATTI	0.1	<	19	<	<	<	<	<	<	<
DPMI	0.1	59	5.3	0.6	6.0	<	588	<	<	<
HHCB	0.1	21054	25630	75	1627	0.4	5.9	77848	44776	6.3
MA	0.1	<	<	<	<	<	<	<	<	<
MK	0.1	0.1	<	<	<	<	0.7	<	0.4	<
MM	0.1	<	<	<	<	<	<	<	<	<
MT	0.1	<	0.5	<	<	<	<	<	<	<
MX	0.1	<	<	<	<	<	0.1	<	<	0.2

sample code TNO		4288-01	4288-02	4280-04	4280-05	3227-06	3227-07	3227-08
sample code Greenpeace		30	31	32	33	35	36	37
sample description	reporting limit	Puma, Jamaica man	Puma, woman	CK one, for men, Eau de Toilette	Jean Paul Gaultier, Classique	Christian Dior, Poison	Calvin Klein, Eternity woman	Chanel No. 5
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
ADBI	0.1	<	<	10	21	nd	nd	nd
AHMI	0.1	<	<	nd	nd	nd	nd	nd
AHTN	0.1	<	1.2	1132	60	20	50	3.2
ATTI	0.1	<	<	30	< 0.5	nd	nd	nd
DPMI	0.1	<	<	< 0.5	< 0.5	nd	nd	nd
HHCB	0.1	0.1	1.4	2709	4902	6248	7992	73
MA	0.1	<	<	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
MK	0.1	<	<	< 0.5	< 0.5	< 0.5	< 0.5	4592
MM	0.1	<	<	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
MT	0.1	<	<	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
MX	0.1	<	<	< 0.5	< 0.5	< 0.5	< 0.5	2.2

Table 3 Macrocyclic musks identified in 29 samples of perfumes. Note that the results are only qualitative.

sample code TNO		4338-01	4338-02	4338-03	4338-04	4338-05	4338-06	4338-07	4338-08	4338-09	4338-10
sample code Greenpeace		1	2	3	4	5	6	7	8	9	10
sample description	identification limit	Adidas, Floral Dream	Aihner, In Leather	Armani, Emporio	Bulgari, BLV Notte	Boss, Hugo Boss	Calvin Klein, Eternity	Celine Dion	Chanel, Chance	Cinéma, Yves Saint Laurent	FCUK, Him
	mg/kg										
muscone	1000							+			
civetone	1000		+								
ambrettolide	1000		+			+					
exaltolide	1000			+		+					+
ethylene brassylate	1000			+	+	+			+	+	+
musconate	1000					+					

sample code TNO		4338-11	4338-12	4338-13	4338-14	4338-15	4338-16	4338-17	4338-18	4338-19	4338-20
sample code Greenpeace		11	12	13	14	15	16	17	18	19	20
sample description	identification limit	Dior, Pure Poison	Gucci, Envy Me	Isabella, Rossellini, My Manifesto	Jean Paul Gaultier, Le Male	Joop, Nightflight	Lancóme, Miracle So Magic!	Melvita, Iris Blue	Naomi Campbell, Sunset	Mexx, Waterlove	Paco Rabanne, Excess pour homme
	mg/kg										
muscone	1000										
civetone	1000									+	
ambrettolide	1000									+	+
exaltolide	1000	+					+		+	+	
ethylene brassylate	1000	+	+	+			+		+		
musconate	1000										

sample code TNO		4338-21	4338-22	4338-23	4338-24	4338-25	4338-26	4338-27	4338-28	4338-29
sample code Greenpeace		21	22	23	24	25	26	27	28	29
sample description	identification limit	Polo, Ralph Lauren Blue	Tommy Hilfiger, True Star	Vanderbilt, Gloria Vanderbilt	Van Gils, Eau De Toilette	Aqua Natural	Bogner, High Speed	The Body Shop, White Musk, Eau De Parfume	Cartier, Le Baiser Du Dragon	Fiorucci Loves You
	mg/kg									
muscone	1000									
civetone	1000									
ambrettolide	1000		+				+			
exaltolide	1000	+					+		+	+
ethylene brassylate	1000						+	+	+	+
musconate	1000									