In-situ Solid-Phase Extraction and Laboratory Analysis of Ultra-trace Synthetic Musks in Municipal Effluent using GC/MS in the Full-Scan Mode

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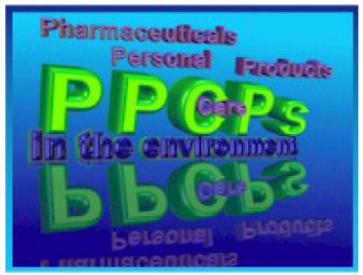
Pharmaceuticals and Personal Care Products (PPCPs) as Environmental Pollutants

◆ *Pollution from Personal Actions, Activities, and Behaviors* ◆



Origins and Fate of PPCPs in the Environment

- About this Site
- Frequently Asked Questions from the Public, Media, and Scientific Communities
- NEW -- Book on Pharmaceuticals in the Environment, from the *American Chemical Society*
- Slide Presentation by Christian Daughton: **Pharmaceuticals & Personal Care Products (PPCPs) as Environmental Pollutants**

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- Critical Review Article by Daughton and Ternes: **Pharmaceuticals & Personal Care Products in the Environment: Agents of Subtle Change?**
  -- Summary/Background
- Origins and Fate of PPCPs in the Environment
- Use of PPCPs in the Environment as Analytical "Tools"

(Continued)

- Multidimensional Science Issues Relevant to Regulatory Considerations
- NEW - "Emerging" Pollutants, and Communicating the Science of Environmental Chemistry and Mass Spectrometry
- Scientific Conferences Devoted to PPCPs in the Environment
- Measurement Prefixes Used in Analytical Chemistry
- Laboratory/Monitoring Research in Environmental Chemistry Branch
- Listing of Scientists Involved with Environmental Aspects of PPCPs
- Opportunities for Funding and for Research in Collaboration with EPA Scientists
- Assistance with Conferences, Seminars, or Lectures
- Relevant Websites
- EPA's Terms of Environment (glossary of technical terminology)
- PPCPs and One Approach of EPA/ORD's to "Emerging" Science Issues

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http://www.epa.gov/nerlesd1/chemistry/pharma/index.htm

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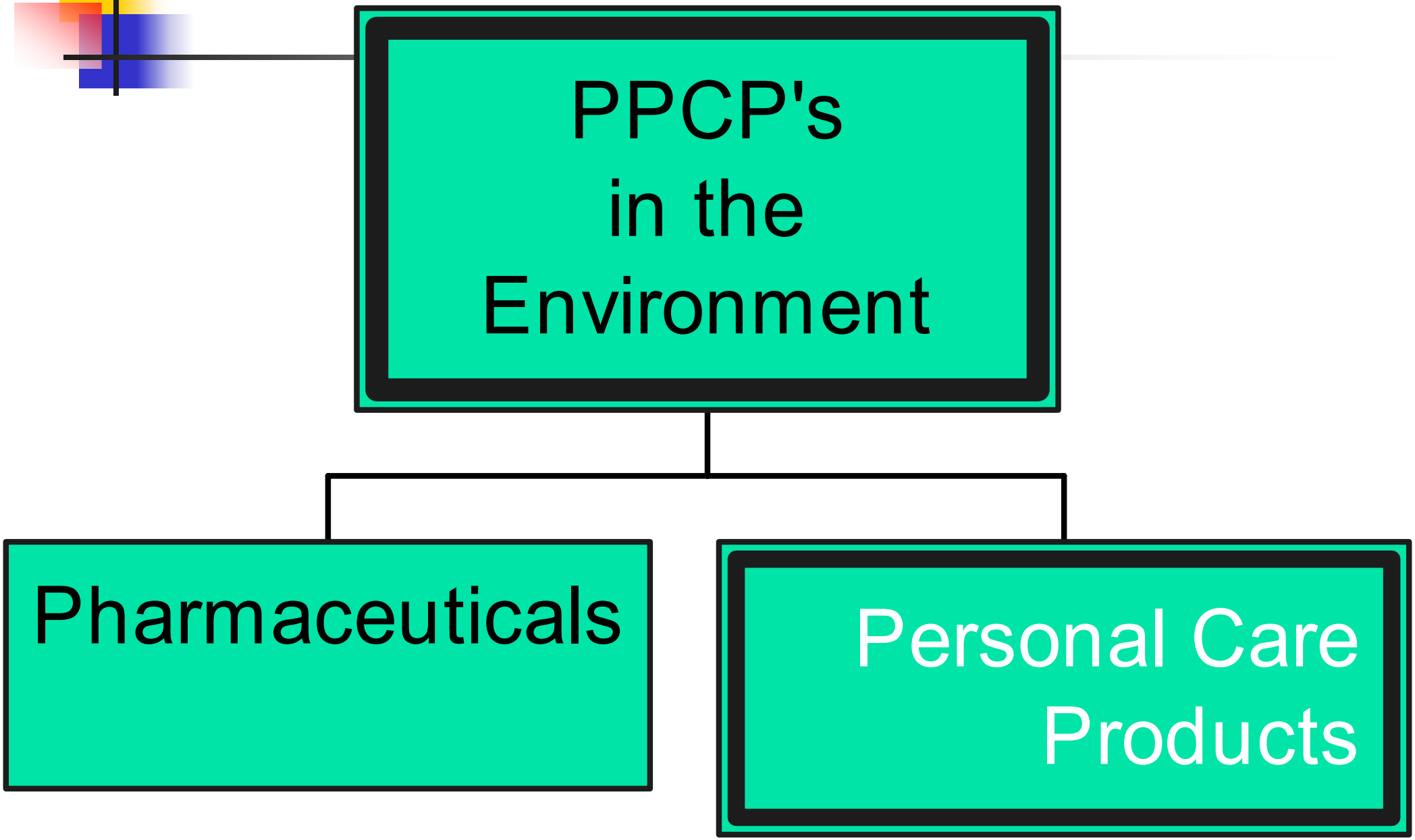
Last Updated: 09/05/01

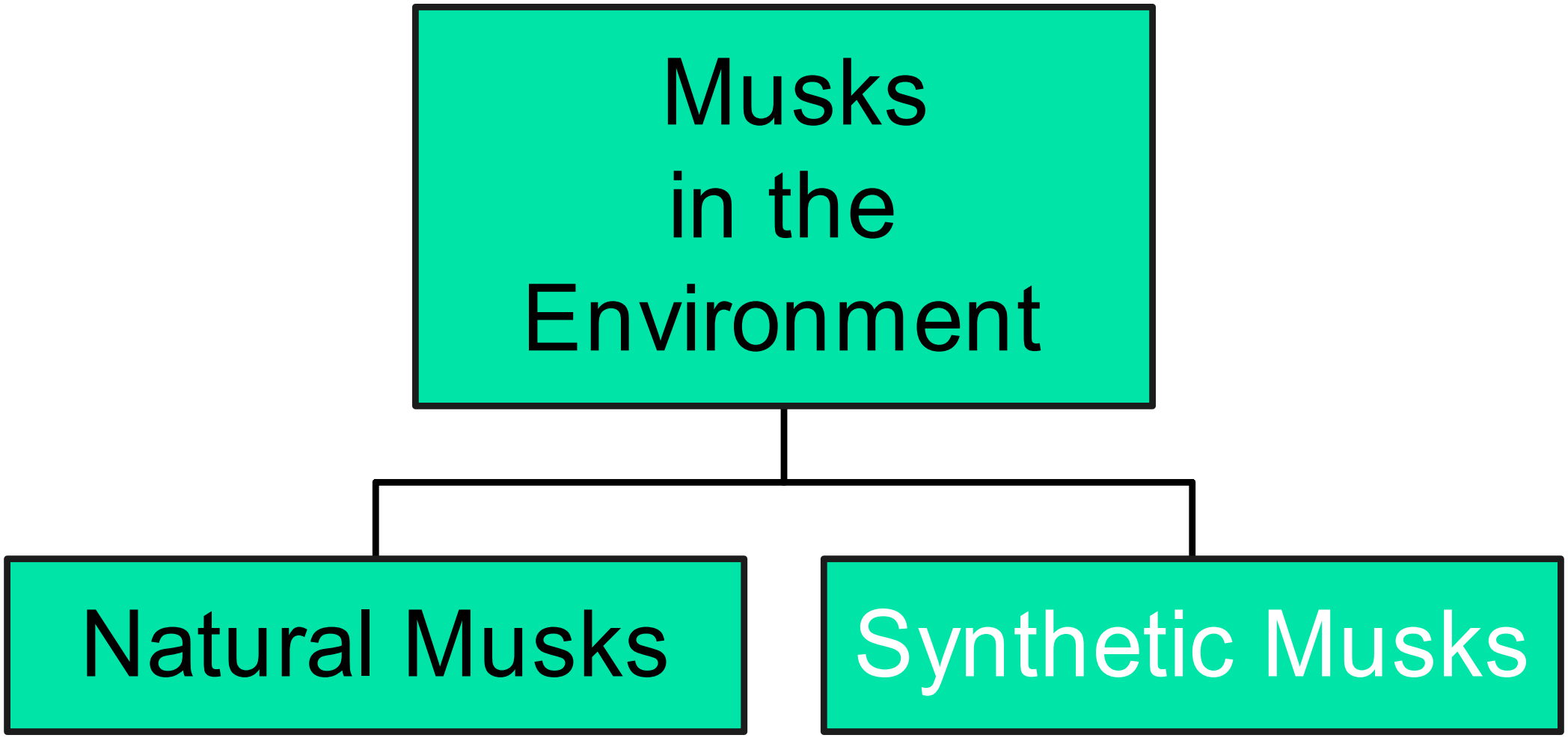
OVERVIEW

- Musks! What are they?
- Why should we care about them?
- How do they get into the aquatic environment?
- Solid phase extraction methodology
- Summary and conclusion

Pharmaceuticals and Personal care Products in the Environment



Fragrance Material



Synthetic musks

- Polycyclic and Nitro musks
- Mimic the strong and persistent aroma of the more expensive natural musks
- Synthesized by the chemical industry as inexpensive substitutes for natural musks.

Uses of synthetic musks

- Additives for perfumes
- Detergents and bath soaps
- Body lotions and hair creams
- Fish baits and herbicides

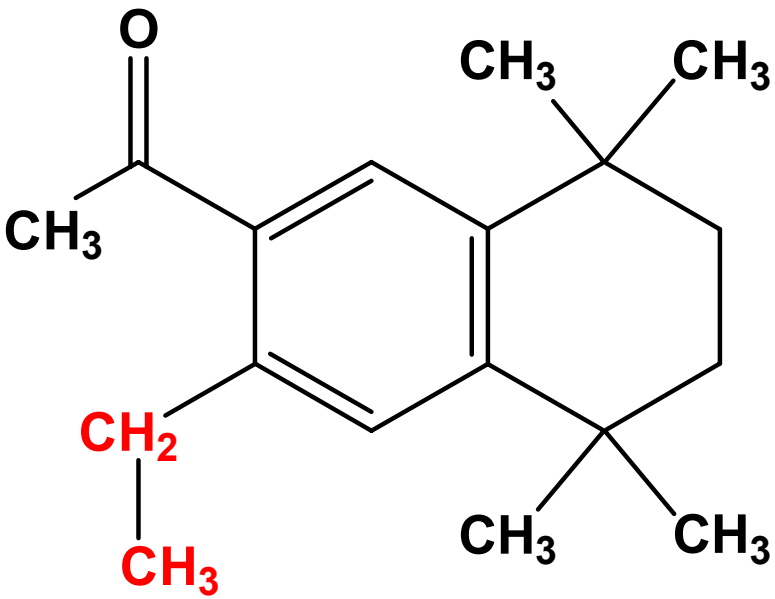
Where are they?

- Sewage influent/effluent. **(Gatermann et al., 1998)**
- Sewage sludge. **(Herren and Berset, 1999)**
- Rivers/Sediments. **(Rimkus et al., 1999)**
- Sediments. **(Fromme et al., 2001)**
- Fish adipose tissue. **(Yamagishi et al., 1981)**
- Human breast milk. **(Rimkus et al., 1994)**
- Human adipose tissue. **(Muller et al., 1996)**

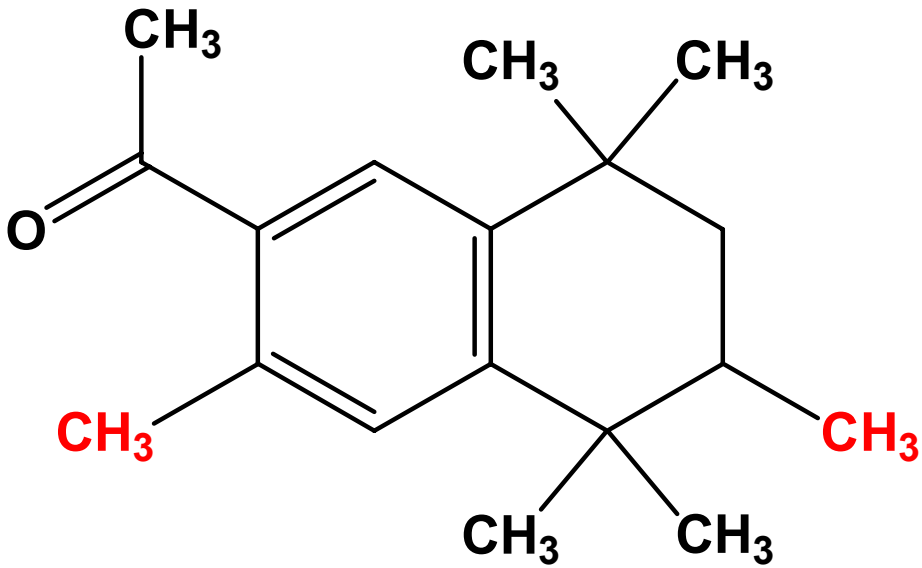
Potential Environmental Hazards

- Bioconcentration in the adipose tissues of aquatic organisms.(Rimkus et al., 1997)
- Musk ambrette is neurotoxic. It causes the paralysis of the hind limbs in the female rats, and atrophy of the testes in the male rats.(Davis, 1967) In 1985, usage stopped by IFRA.
- In 1977, Versalide was voluntarily removed from the market by the Fragrance Industry due to its discoloration of internal organs (Opdyke, 1979), demyelination of PNS and CNS, and cumulative neurotoxicity in rats.(Spencer et al., 1979)

Chemical Structures of Synthetic Polycyclic Musks



Versalide®

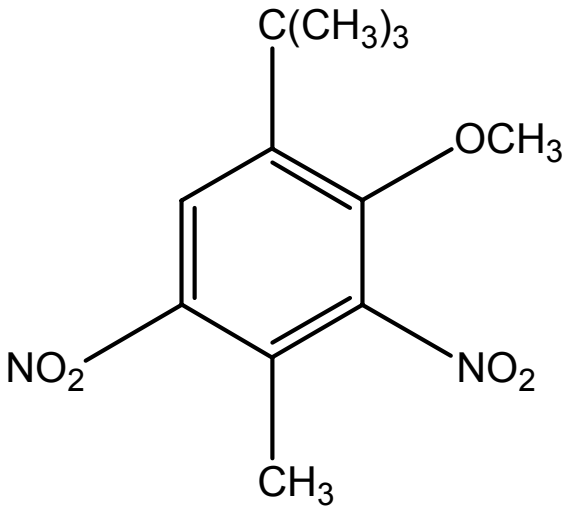


Tonalide®

Chemical Structures of Synthetic Nitro Musks

$C_{12}H_{16}N_2O_5$
Mol. Wt.: 268.26880

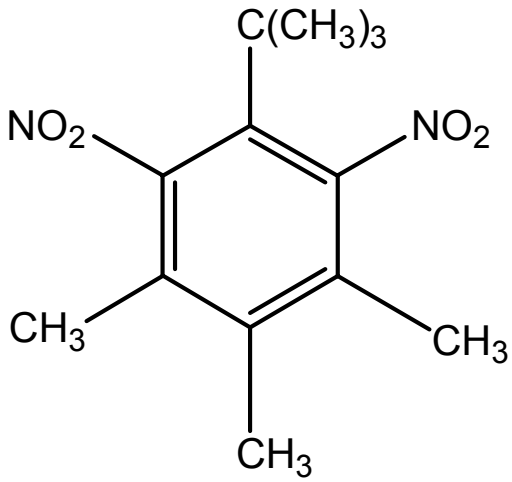
2,6-dinitro-3-methoxy-4-tert-butyl toluene



Musk ambrette

$C_{13}H_{18}N_2O_4$
Mol. Wt.: 266.29620

1-tert-butyl-2,6-dinitro-3,4,5-trimethylbenzene

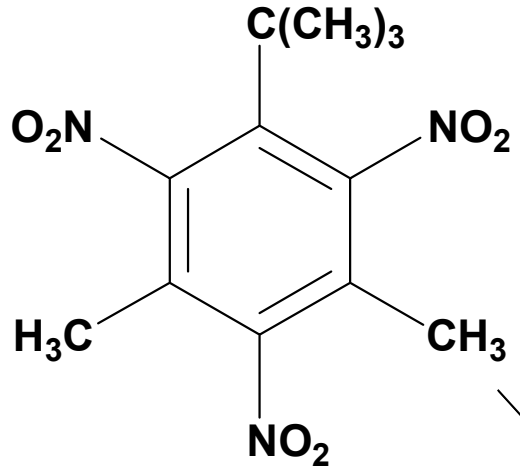


Musk tibetene

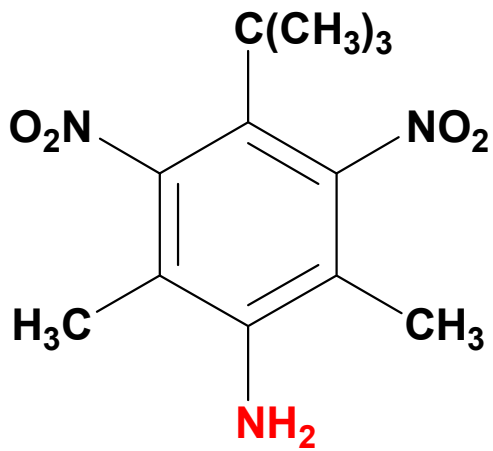
Chemical Structures of Musk Xylene and Metabolites

(Musk Xylene)

1-*tert*-Butyl-3,5-dimethyl-2,4,6 -trinitro-benzene

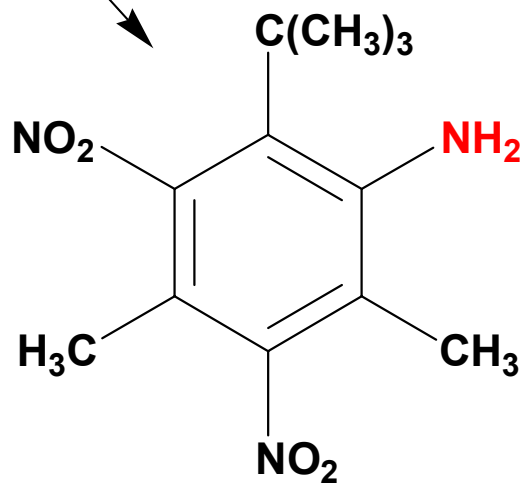


Nitro-reductase enzymes



(4-AMX)

4-*tert*-Butyl-2,6-dimethyl-3,5-dinitro-phenylamine



(2-AMX)

2-*tert*-Butyl-4,6-dimethyl-3,5-dinitro-phenylamine

Qualitative and Quantification

- To perform human health risk assessment for these compounds, we need tools to identify and quantify them in their various environmental compartments.

Previous studies

- About 30 years ago, Junk et al*., Muskty, and Nickless pioneered the use of SPE for direct extraction/enrichment of environmental samples.
- Early studies focused on Amberlite XAD-2 & 4.
- In 1979, Rees and Au**, used methanol/XAD-2 slurry for good recoveries of pesticides.
- In 2001, we evaluated several sorbents and identified poly(methylmethacrylate) mixed 1:1 with poly(styrene-divinylbenzene) as a superior sorbent for for the extraction of synthetic musks.
- Commercially available as *Varian Abselut NEXUS*

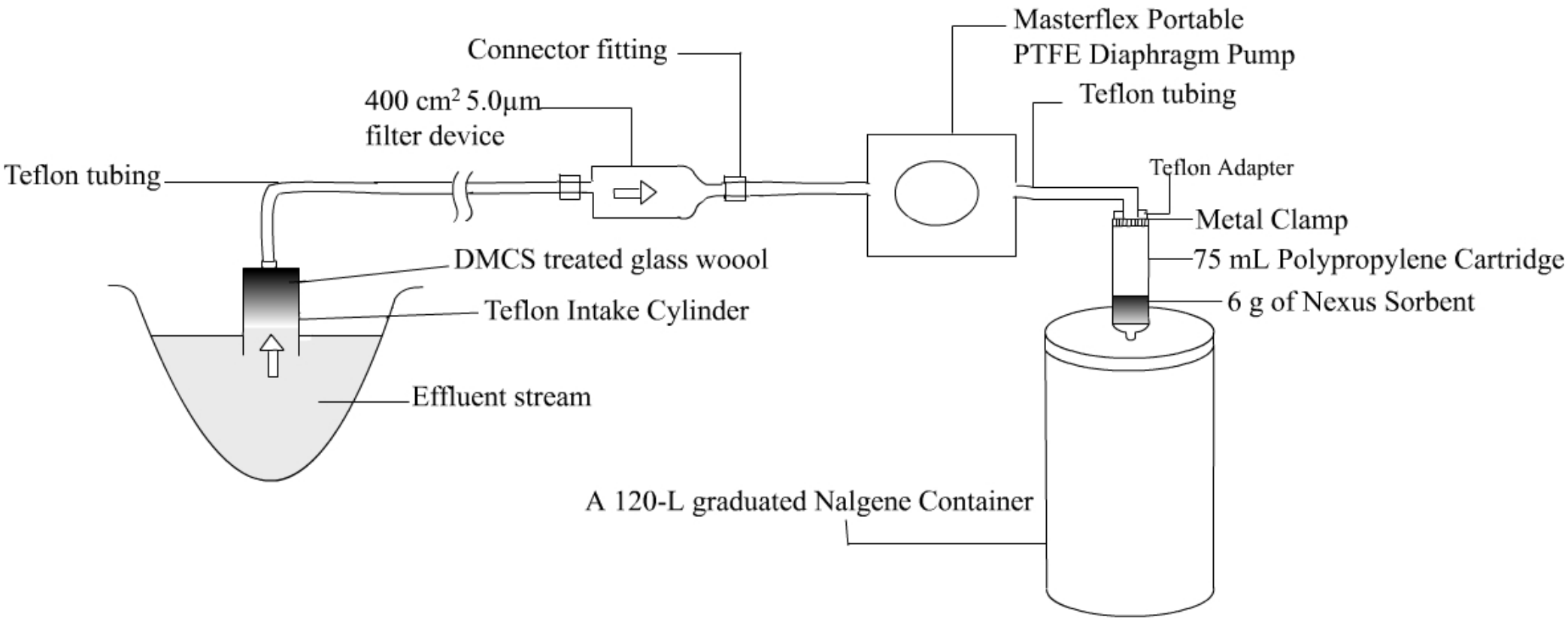
*G.A. Junk, J.J. Richard, M.D. Grieser, D. Witiak, J.L. Witiak, M.D. Arquello, R.Vick, H.J. Svec, J.S. Fritz, G.V. Calder, J. Chromatogr. 199 (1974) 745.

**V. Rees, L. Au, Bulletin of Environmental Contamination and Toxicology. 22 (1979) 561

Recent Studies of Others

- Synthetic musks are hydrophobic (Log K_{ow} 4.3 to 6.3)
- Liquid-liquid extraction of unfiltered samples yielded higher concentration in surface water
- Select (high industry volume) musks compounds were extracted with silica disk.

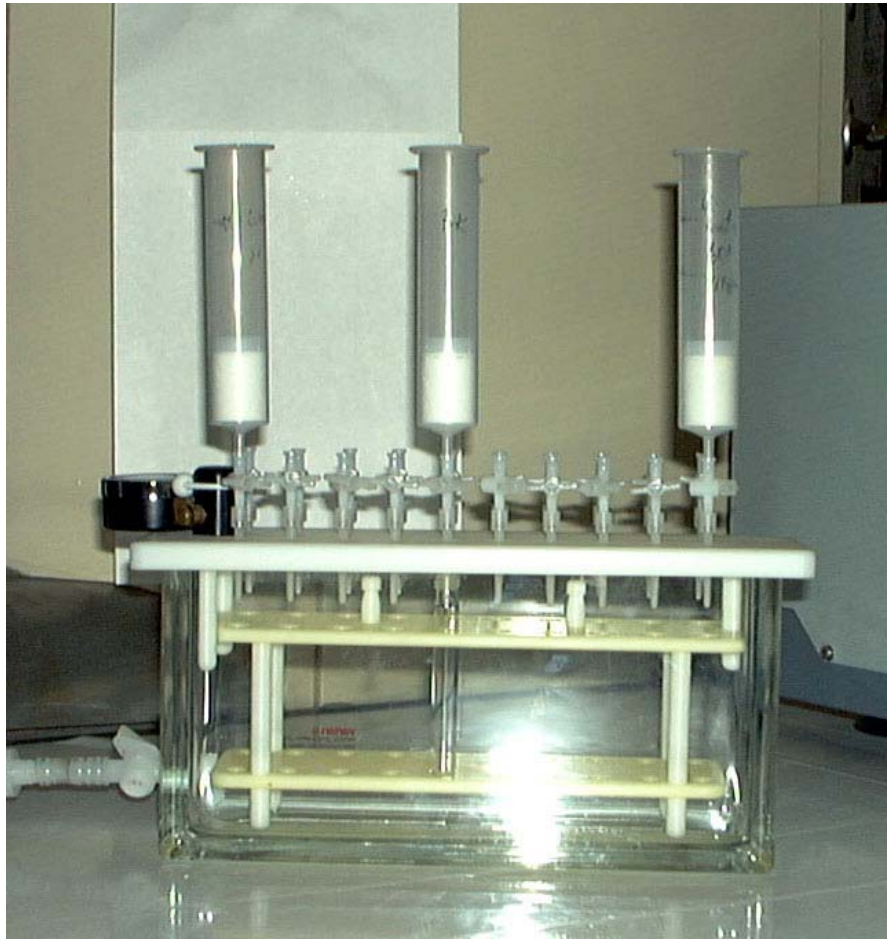
Fig. 1 **On-Site Solid-Phase Extraction Assembly**



In-situ Extraction of Synthetic Musk Compounds

Elution of Analytes

- 60 mL hexane
- 60 mL ethyl acetate
- Vacuum at 252 mm Hg

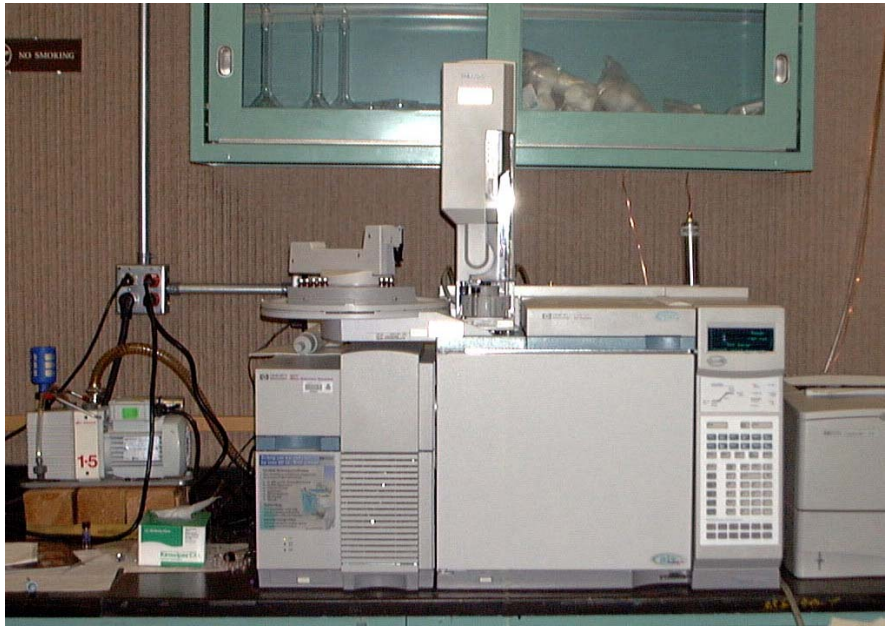


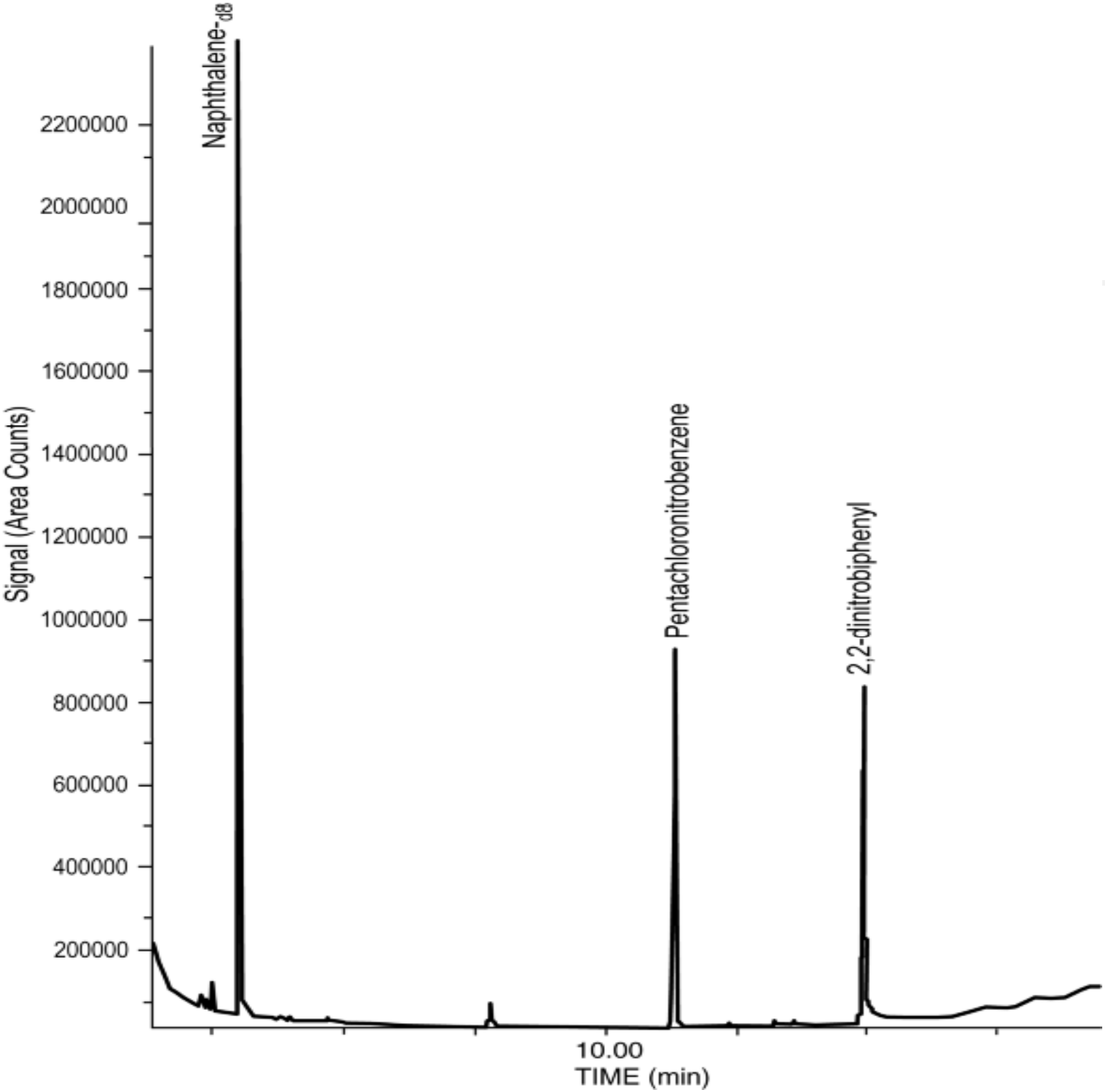
Turbo-vap™ Solvent Exchanged

- Temperature = 30°C
- Chiller = 10°C
- Endpoint = Sensor
- Fan = 4000 rpm
- 0.4 mL, add 1 mL toluene

GC/MS Analysis

- Injection port =250°C
- 90°C for 1min, ramp at 10°C to 300°C, hold for 5 min.
- Interface =300°C.
- 2 µL sample injected.




Naphthalene-d8
Pentachloronitrobenzene
2,2-dinitrobiphenyl
Signal (Area Counts)
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
10.00
TIME (min)

Ions used.

TABLE 4. CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS

Compound	Retention Time (min)	Primary Ion	Secondary Ion (s)
Naphthalene-d^8 (I.S.)	4.33	**136.10**	135.10, 137.10, 108.10
Cashmeran	8.01	**191.10**	192, 135.10, 107
Celestolide	10.46	**229.10**	224.10, 173, 230.1
Phantolide	10.91	**229.10**	224.10, 187.10, 230
Pentachloronitrobenzene (Surr.)	11.06	**236.80**	294.80, 213.8, 265
Versalide	11.45	**243.20**	244.20, 258.20, 259.20
Musk ambrette	11.69	**253.00**	268.00, 254.00, 251.00
Traseolide	11.84	**215.10**	216.10, 173.00
Galaxolide	11.89	**243.10**	258.10, 213.10, 244.00
Tonalide	12.00	**243.10**	258.10, 244.00, 201.10
Musk xylene	11.99	**282.00**	297.10, 283.10
Musk moskene	12.23	**263.10**	278.10, 264.00, 221.00
MuskTibetene	12.76	**251.00**	266.10, 252.00, 115.00
Musk ketone	13.17	**279.00**	294.10, 128.00, 280
2,2-dinitrobiphenyl (Surr.)	13.95	**198.00**	168.00, 139.00, 115.00
2-Amino musk xylene	14.51	**264.10**	249.10, 215.10, 191.00
Amino musk ketone	14.36	**267.10**	252.10, 218.10, 160.10
4-Amino musk xylene	14.92	**252.00**	267.10, 218.00, 235.00

I.S. = internal standard

Surr. = Surrogate

Table 5. % Spike Recovery data from 60 L Sample* (n=3)

Analytes	Nanopure water (%RSD)	STP Effluent (%RSD)	MDL (ng/L)
Musk xylene	102 (4)	97 (6)	0.02
Musk ketone	98 (3)	95 (5)	0.20
Musk ambrette	101 (5)	96 (7)	0.30
Musk moskene	96 (4)	92 (4)	0.03
Musk tibetene	98 (2)	95 (7)	0.02
Versalide® (AETT)	99 (5)	96 (6)	0.02
Galaxolide® (HHCB)	99 (3)	97 (5)	0.02
Phantolide® (AHDI)	97 (3)	91 (5)	0.02
Cashmeran® (DPMI)	99 (4)	94 (6)	0.02
Celestolide® (ADBI)	98 (3)	96 (8)	0.02
Traseolide® (ATII)	95 (5)	90 (8)	0.02
Tonalide® (AHTN)	107 (3)	94 (6)	0.02
4-Amino musk xylene	87 (5)	80 (10)	0.30
2-Amino musk xylene	89 (7)	82 (11)	0.25
Amino musk ketone	90 (6)	92 (8)	0.25

* Recovery data from extraction through GPC steps.

Table 6. Concentrations (ng/L) of synthetic musk compounds, and nitro musk metabolites in STP effluent stream.

Analytes	85 L[a]	65 L[a]	85 L[b]	45 L[b]	60 L[c] (%RSD)
Musk xylene	**1.3**	< MDL	< MDL	**0.5**	< MDL
Musk ketone	**27.5**	**21.5**	**23.4**	**21.3**	< MDL
Musk ambrette	< MDL	< MDL	< MDL	< MDL	< MDL
Musk moskene	< MDL	< MDL	< MDL	< MDL	< MDL
Musk tibetene	< MDL	< MDL	< MDL	< MDL	< MDL
Versalide	< MDL	< MDL	< MDL	< MDL	< MDL
Galaxolide	**138**	**111**	**152**	**35.0**	**40.8 (1.8)**
Phantolide	**4.3**	**3.1**	**5.0**	**2.5**	**2.4 (4.3)**
Cashmeran	< MDL	< MDL	< MDL	< MDL	< MDL
Celestolide	**2.1**	**0.3**	**0.3**	**0.5**	**1.4 (7.2)**
Traseolide	**83.8**	**34.5**	**126**	**6.6**	< MDL
Tonalide	**67.3**	**47.1**	**92.2**	**26.6**	**36.8 (2.5)**
4-Amino musk xylene	**1.4**	**11.6**	< MDL	**31.5**	< MDL
2-Amino musk xylene	< MDL	< MDL	**0.9**	< MDL	< MDL
Amino musk ketone	< MDL	< MDL	< MDL	< MDL	< MDL

[a] Effluent sample downstream from a tertiary sewage treatment plant's discharge pipe. (n=1)
[b] Effluent sample taken 14 days later from same location. (n=1)
[c] Effluent sample near a different tertiary sewage treatment plant's discharge pipe. (n=3)

Summary

- Enrichment factor approaches 10^6 : 1, without methanolic pretreatment or activation of resin
- This simplified process leads to the extraction of dissolved-phase analytes only (particulates > 5.0 µm excluded)
- Resultant sample is highly compact, easily stored, and transported
- High signal/noise enables unknown to be authenticated with NIST mass spectral library
- Structures are easily proven by showing identity to authentic standards (GC- ret. time and full mass spectrum)

Osemwengie, L.I., and S. Steinberg. On-Site Solid Phase Extraction and Analysis of Ultra-trace Synthetic Musks in Municipal Sewage Effluent Using Gas Chromatography-Mass Spectrometry, Full-scan Mode. "Journal of Chromatography A", Vol 932/1-2, pp 107-118 (2001).

Acknowledgement

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