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Levels of Synthetic Musks in Municipal Wastewater for Estimating Biota Exposure in Receiving Waters

**A Doctoral Dissertation Proposal by Lantis Osemwengie
Personal Care Products in the Environment
February 2000**

Abstract

Natural musk (muscone) is a highly prized natural product for use in expensive consumer fragrances. Inexpensive synthetic substitutes have been commercialized over the last 40 years. Two main chemical classes are used commercially -- the nitro musks (which fulfilled the original demand for inexpensive fragrance additives for personal care products such as bath soap, body lotions, perfumes, and washing detergents) and the newer polycyclic musks which have musklike smell. The commercial nitro musks include musk ketone, musk moskene, musk ambrette, musk xylene, and musk tibetene. The polycyclics include trade named compounds such as Galaxolide[®], Tonalide[®], Celestolide[®], Traseolide[®], Phantolide[®], and Cashmeran[®], among others. These consumer chemicals are manufactured and consumed in very large quantities worldwide. They are volatile, not amenable to normal environmental transformations, and lipophilic—all traits that should ordinarily lead to their being characterized as persistent, bioaccumulative, toxic chemicals (PBTs).¹⁶ Their use leads to their entry into the city sewage systems (presumably from bathing and other washing activities), and then into the aquatic ecosystem, where they can bioconcentrate and bioaccumulate in the tissues of aquatic organisms. Given their lipophilic bioaccumulative nature and possibly worldwide environmental distribution, they have the potential to be regarded as ubiquitous environmental contaminants.

Statements of the Problem

Certain synthetic musks have been categorized as carcinogenic chemicals.^{1,2} Musk ambrette, musk xylene and musk ketone are known to exhibit mutagenic and genotoxic properties.³ Musk xylene and musk ketone are strong inducers of xenobiotic toxifying and detoxifying enzymes in rat liver.³ Musk ambrette, a recognized neurotoxicant in rats, has also been shown to cause rat testicular atrophy.^{4,5,6,7} Growth retardation and progressive paralysis of the hind limbs in laboratory rats have resulted from subchronic feeding of musk ambrette.⁸ Mersch-Sundermann et al. (1996), identified a DNA damaging effect which resulted from synergism of enzyme inducers, i.e., musk xylene and musk ketone, and pre-genotoxicants, i.e. Benzo-[a]-pyrene and 2-aminoanthracene.⁹

Worldwide annual production of nitro musks and polycyclic musks in 1988, was estimated at 7000 metric tons,¹⁰ while the annual worldwide production volume of polycyclic musks in 1999 was 6000 tons/year.¹¹ Approximately 1,000 tons per year of musk xylene is consumed worldwide through the use of detergents.¹² In 1987, the estimated worldwide synthetic musk market was valued at \$215 million, \$60 million of it was the U.S. market value. Musk xylene and musk ketone are apparently ubiquitous in distribution. In 1981, Yamagishi et al, reported the identification of musk xylene and musk ketone in freshwater, marine biota, and water samples from Tama River in

Tokyo.^{13,21} The levels of musk xylene and musk ketone in freshwater fish were reported to be 0.20 and 0.05 ppm on wet weight basis, respectively.²¹ These levels were an order of magnitude higher than some pesticides (dieldrin 0.011 ppm and 4,4-DDE 0.015 ppm) in the same samples.²¹ The consumption of fish and drinking water as well as the use of personal care products could lead to the ingestion of these substances in humans.³ In 1981, nitro musk residues were identified in human adipose tissues and in human milk indicating their high lipophilic tendency and persistence.¹⁴ Nitro musk gets into human fat tissues through alimentary intake and dermal sorption from cosmetics and detergent.¹⁵ Musk xylene concentrations in human adult adipose tissue and milk samples ranged from 0.01 to 0.25 mg/kg fat.¹⁵ The concentrations of musk xylene found in the fat tissues of children and newborns were up to 0.6 mg/kg fat.¹⁵ Musk ketone is highly toxic to aquatic organisms; LC₅₀ value in 28 hr for carp is 5.1 ppm.¹⁹ According to a recent review article,¹⁶ synthetic musks may be members of a larger group of xenobiotics that may insidiously affect aquatic species and initialize subtle changes.

Due to the large worldwide production and consumption of synthetic musks, and their chemical and ecological characteristics such as lipophilic/bioconcentration tendency and persistence, synthetic musks deserve closer scrutiny as a new class of environmental contaminants.

Hypothesis and Objective

The objectives of this research require the development of a method for the simultaneous analysis of polycyclic and nitro musks (and their transformation products, if any) in surface water, sediments, and fish (carp, *Cyprinus carpio*), in the effluent to, and receiving water from, a major metropolitan sewage treatment plant. Transport and kinetics models will be developed to describe the relationship between concentration in the discharge effluent and those in the fish. The methods developed from this research may be used for exposure assessment.

Synthetic musks, specifically nitro musks and polycyclic musks in municipal receiving waters and their concentrations in the biota, are functions of their levels in the commingled municipal wastewater discharged.

Background

By filtering dissolved and suspended food from their aquatic environments, some aquatic organisms inescapably suffer from continual exposure to xenobiotic toxicants such as synthetic musks. The extent of exposure may be determined by measuring levels of synthetic musks from their potential source (communal sewage effluent). Synthetic polycyclic compounds [Table 1; Galaxolide[®](1), Tonalide[®](2), Celestolide[®](3), Cashmeran[®](4), Phantolide[®](5), and Traseolide[®](6)] are derivatives of pyran(1), tetralin(2) and indane(3,4,5,6), while nitro musk compounds [Table 2; musk ketone, musk moskene, musk ambrette, musk tibetene and musk xylene] belong to a group of di- and trinitrobenzene derivative compounds with musk-like odor. One of the major pathways of biological transformation of nitroaromatic compounds is the reduction of the nitro group to the corresponding amino group (Table 3). These latter concentrations are 4 to 40 times higher than the parent compounds.¹⁶

Table 1.

Chemical structures, Trade names, CAS names and Registry Numbers, molecular weights, and formulas of six polycyclic musk compounds.

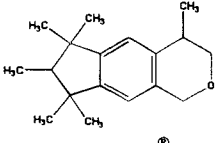
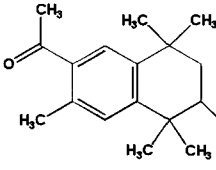
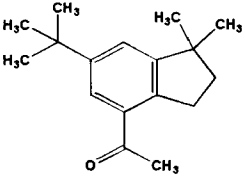
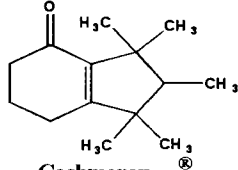
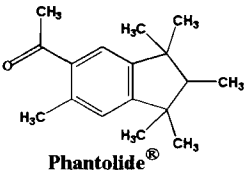
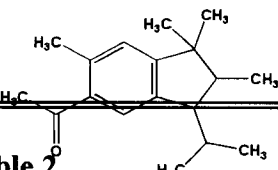
| Chemical structure and Trade name | CAS Name (Acronym) | CAS No. and Molecular Weight | Molecular formula |
|--|---|------------------------------|-----------------------------------|
|  <p>Galaxolide®</p> | 1,3,4,6,7,8-hexamethylcyclopenta-[g]-2-benzopyran (HHCB) | 1222-05-5 258.40 | C ₁₈ H ₂₆ O |
|  <p>Tonalide®</p> | 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethylnaphthalenyl)ethanone (AHTN) | 1506-02-1 258.40 | C ₁₈ H ₂₆ O |
|  <p>Celestolide®</p> | 1-[6-(1,1-dimethylethyl)-2,3-dihydro-1,1-dimethyl-1H-inden-4-yl]ethanone (ADBI) | 13171-00-1 244.38 | C ₁₇ H ₂₄ O |
|  <p>Cashmeran®</p> | 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one (DPMI) | 33704-61-9 206 | C ₁₄ H ₂₂ O |
|  <p>Phantolide®</p> | 1-(2,3-dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl)ethanone (AHDI) | 15323-35-0 244.38 | C ₁₇ H ₂₄ O |
|  <p>Traseolide®</p> | 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methyl-ethyl)-1H-inden-5-yl]ethanone (ATII) | 68140-48-7 258.4 | C ₁₈ H ₂₆ O |

Table 2. Chemical structures, IUPAC names, and CAS Registry Numbers, molecular weights, and formulas of five nitro musk compounds.

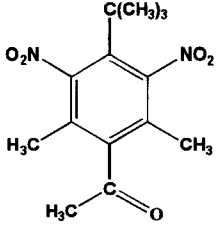
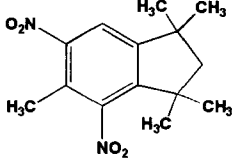
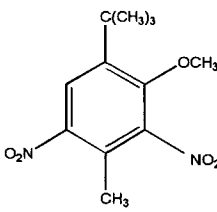
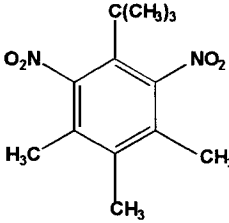
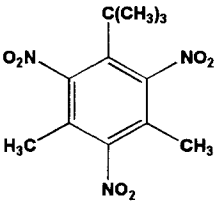
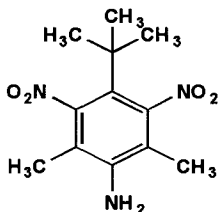
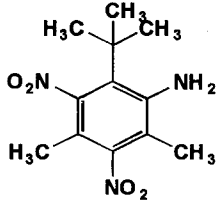
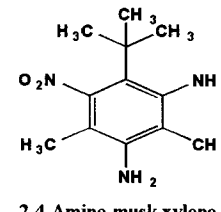
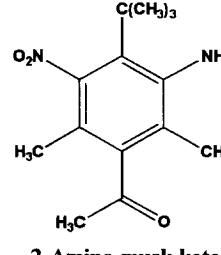
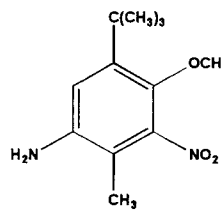
| Chemical structure and Common Name | IUPAC Name and No. | Molecular Weight | Molecular formula |
|--|--|------------------|----------------------|
|  <p>Musk ketone</p> | <p>1-tert-butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene (MK)</p> <p>81-14-1</p> | 294.31 | $C_{14}H_{18}N_2O_5$ |
|  <p>Musk muskene</p> | <p>4,6-dinitro-1,1,3,3,5-pentamethylindane</p> <p>116-66-5</p> | 278.31 | $C_{14}H_{18}N_2O_4$ |
|  <p>Musk ambrette</p> | <p>2,6-dinitro-3-methoxy-4-tert-butyl toluene</p> <p>83-66-9</p> | 268.27 | $C_{12}H_{16}N_2O_5$ |
|  <p>Musk tibetene</p> | <p>1-tert-butyl-2,6-dinitro-2,4,5-trimethylbenzene</p> <p>145-39-1</p> | 266.30 | $C_{13}H_{18}N_2O_4$ |
|  <p>Musk xylene</p> | <p>1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene</p> <p>81-15-2</p> | 297.27 | $C_{12}H_{15}N_3O_6$ |

Table 3
Chemical structures, IUPAC names, CAS Registry Numbers, molecular weights, and formulas of five nitro musks transformation products

| Chemical structure and abbreviated name | CAS Name (Abbreviation) | CAS No. and Molecular Weight | Molecular formula |
|--|---|------------------------------|---|
|  <p>4-Amino-musk xylene</p> | 4-Amino-1-tert-butyl-3,5-dimethyl-2,6-dinitrobenzene (4-NH ₂ -MX) | 107342-55-2 267.27 | C ₁₂ H ₁₇ N ₃ O ₄ |
|  <p>2-Amino-musk xylene</p> | 2-Amino-1-tert-butyl-3,5-dimethyl-4,6-dinitrobenzene (2-NH ₂ -MX) | 107342-67-6 267.27 | C ₁₂ H ₁₇ N ₃ O ₄ |
|  <p>2,4-Amino-musk xylene</p> | 2,4-Diamino-1-tert-butyl-3,5-dimethyl-6-nitrobenzene (2,4-di-NH ₂ -MX) | N/A 237.27 | C ₁₂ H ₁₉ N ₃ O ₂ |
|  <p>2-Amino-musk ketone</p> | 2-Amino-1-tert-butyl-3,5-dimethyl-6-nitro-4-acetylbenzene (2-NH ₂ -MK) | N/A 264 | C ₁₄ H ₂₀ N ₂ O ₃ |
|  <p>6-Amino-musk ambrette</p> | 6-Amino-2-nitro-3-methoxy-4-tert-butyltoluene (6-NH ₂ -MA) | N/A 238.27 | C ₁₂ H ₁₈ N ₂ O ₃ |

Dissertation proposal

My proposed research will focus on the levels of synthetic polycyclic musks, synthetic nitro musks, and their transformation products in the suspected source, Las Vegas commingled

municipal sewage treated effluent, the aquatic ecosystem where they bioconcentrate and bioaccumulate, and the adipose tissues of one of the top predators, (carp, *Cyprinus carpio*) in the aquatic ecosystem. By determining the average concentrations of these compounds in the effluents from the city sewage water reclamation and treatment plants, possible exposure of aquatic organisms down stream may be estimated.

Research Design and Methods

Field studies will be conducted from December 2000 through May 2001 at the Las Vegas Wash site, and Lake Mead in Southern Nevada. This research will study the levels of synthetic musk in Municipal wastewater effluent, bioconcentration and bioaccumulation of synthetic musk in the adipose tissues of fish, levels of synthetic musk in Lake Mead sediment samples. Representative samples will be taken at the estuary where the wash stream discharges xenobiotics loading to the Lake. Multiple regression statistical analysis will be used to analyze the data so collected.

$$Y = B_0 + B_1x_1 + B_2x_2$$

X_1 = Concentration of synthetic musk in water

X_2 = Concentration of synthetic musk in sediment

Y = Concentration of synthetic musk in fish

B_0 = Y intercept

B_1, B_2 = Constants.

Chemicals

Synthetic musks (99% purity) were purchased from Promochem (Wesel,

Germany). Substantial quantities of musk ketone, musk ambrette and musk xylene were kindly provided by the Institute of Food Chemistry, University of Hohenheim (Stuttgart, Germany). Organic solvents; n-hexane (optimal grade), Methylene Chloride (HPLC grade), Ethyl acetate, Diethyl ether, Petroleum ether, Acetone, Methanol (HPLC grade), were purchased from Lancaster, USA. Hydrazine hydrate and Raney nickel (slurry) were purchased from Aldrich, Milwaukee, Wisconsin USA.

Synthesis of nitro musk transformation products

About 500 mg of musk ketone, musk xylene and musk ambrette will be synthesized using Hydrazine hydrate as reducing agent, in the presence of slurry Raney nickel, as reported by Gatermann et al, (1998).

Purification of transformation products by thin layer chromatography (TLC)

Purification and separation of 6-amino-musk ambrette, 4-amino musk xylene and 2-amino musk ketone from their respective reaction mixtures will be done by using preparatory TLC plates and eluted with a 1:1 mixture of n-hexane and ethylacetate.¹⁸

Sample collection

The number of samples to be collected will be determined by collecting ten experimental samples of effluent wastewater, fish (carp, *Cyprinus carpio*), and Lake Mead sediment for the determination of standard deviations. With a calculated standard deviation, an acceptable error value can be used to obtain the minimum number of samples needed to achieve a specific confidence level.

$$n = \left(\frac{s \cdot t}{E} \right)^2$$

n= number of samples
s= sample standard deviation

t= normal deviation student t-table, at the 95% probability

E= acceptable error in ppb

This proposed research involves the collection of effluent wastewater samples, fish (carp, *Cyprinus carpio*) of approximately equal length and weights from several sites of Lake Mead, and sediment samples from the same sites where the fish were taken. Municipal effluent and sediment samples will be collected from the municipal treatment plant, and the stream between the municipal discharge and Lake Mead. To incorporate the month-to-month and seasonal variation of the concentration of synthetic musk, due to sorption, sedimentation, photochemical and biological degradation, the experimental design will require the process of sample collection to be carried out on a monthly basis for as long as six months. The fish usually avoid the hot Lake waters during the hot summer months, and as such, the summer months will be excluded. Since there is little or no rainfall in the desert, rainfall data will not be collected. Collaboration with USGS and/or any other organization would be most useful in this regard.

Instrumentation

The necessary instruments for the analysis of samples already exist at the U.S. EPA. This includes Accelerated Solvent Extraction (ASE) for solid-sample extraction, Accu*prep 7000 SPE manifold (CPI international, Santa Rosa, CA. USA). Turbo Vaporization 500 Workstation (Zymark Corporation, Hopkinton, MA, USA), for concentrating solvents, Gel Permeation Chromatography (Waters Corporation, Milford, MA. USA), for oil and sulfur removal, GC/EI/MS, GC/NCI/MS (Agilent Technologies, San José, CA, USA). GC/HRMS (Finnigan MAT 900, San José, CA, USA), for identifying ion compositions produced from unidentified compounds. Camag TLC Scanner II with Deuterium lamp (Camag Scientific Muttenz, Switzerland), for scanning by absorbance and fluorescence, Fluorescence analysis Cabinet (Spectronics Corporation, Westbury, New York, USA), with illumination at 254 and 366 nm, for viewing developed TLC plates.

University of Nevada Las Vegas, will provide Portable Master Flex Sampling Pump (Cole-Parmer Instrument Company, Barrington, IL, U.S.A), for pumping aqueous samples through XAD-2 cartridges (Altech, Deerfield, IL, U.S.A).

Extraction and Cleanup

To reduce the volume of solvent used, aqueous samples will be extracted with CPI

Accu*prep 7000(C₁₈) solid phase extraction manifold speed disks with 47-mm membranes (Bakerbond), using 20 ml methylene chloride (pesticide grade) per liter of aqueous sample as the eluting solvent.²² Synthetic musks are relatively non-polar. Their biotransformation products are more polar. To effectively extract the synthetic musks and their transformation products from fish adipose tissues, a mixture of non-polar and polar solvents will be used. 50/50 (V/V) n-hexane and acetone. The fish adipose tissues and sediment samples will be completely homogenized and extracted by automated ASE.

Clean-up of the extracted solid samples will be done by GPC, fitted with a 515 HPLC pump, 717 Plus autosampler, fraction collector, UV detector (254nm), two Envirogel[®] columns (19mm X 300mm and 19 X 150mm), and Envirogel[®] guard column (4.6 X 30mm). A 1 g silica gel column will be used to isolate synthetic musks from the undesired components. This will be done by eluting 0.5 ml volume of extract in n-hexane with 15 ml of methylene chloride.²²

Sample Analysis

The analyses of polycyclic musks and nitro musks in fish, sediment and water samples will be carried out with GC/EI/MS and GC/NCI/MS systems (6890 gas chromatograph-5973 mass selective detector), each equipped with an HP-5 MS column (30 m x 0.25 mm I.D., 0.25- μ m film thickness), HP-Chem acquisition software, NIST-98 environmental samples spectral library and Pflieger MS Drug Spectral Library. If necessary, elemental compositions of molecular ions (M⁺) and key fragment ions of some of the biotransformation compounds will be determined with GC/HRMS.

Expected Results and Application

From the proposed research, we will be able to determine the concentration of synthetic and polycyclic musks in the Las Vegas Wash water, and sediments, Lake Mead sediments and water, and fish from Lake Mead. The data collected from this research may be used for environmental risk assessment in the Southern Nevada area.

Estimated cost to UNLV

Although the cost of this research has largely been born by U.S.EPA, UNLV nevertheless, will still need to bear the cost of sample collection. This may be deflected by collaboration with Professor Jacimaria Batista who already has a 24 hour time proportional automatic sampler in place at the Clark County Water Treatment Plant. Due to the labor intensive nature of this research, funds would be needed to help pay for fish and sediment sample collection by UNLV undergraduate students, or any other reliable sources. UNLV may need to contract out this part of the project. About \$2000.00 will be needed to supplement funds from U.S. EPA for chemical standards and GC/MS consumables.

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