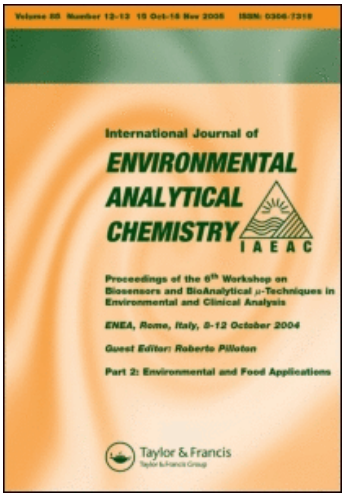

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Identification of several Tonalide® transformation products in the environment

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Organic synthesis has been applied to detect potential Tonalide (AHTN) transformation products in a semi-quantitative way in environmental samples by using a well-established trace-analytical method, developed for the quantification of the parent compound AHTN. For the first time, indications for the presence of transformation products of the synthetic musk compound 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)ethanone (**1**) (Tonalide®, AHTN) have been found in the environment. Pure standards of the detected products were obtained by multistep syntheses from Tonalide®. Two main derivatives, 3-acetyl-5,6,7,8-tetrahydro-5,5,7,8,8-pentamethyl-2-naphthalenecarbaldehyde (**2**) and (3-ethyl-5,6,7,8-tetrahydro-5,5,7,8,8-pentamethyl-2-naphthalenyl)methanol (**4**), and three minor products, (5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphtalenyl)methanol (**6**), 5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphtalenecarbaldehyde (**7**), and methyl 5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenecarboxylate (**8**), were identified in three human breast-milk samples and three fish samples. All samples contained one or several of these transformation products at a concentration range of around 10 pg g^{-1} wet weight.

Keywords: Tonalide; Transformation products; Breast milk; Fish; Gas chromatographic analyses; GC/MS

1. Introduction

Synthetic musks are a group of persistent organic chemicals that have been used as fragrances in household products, perfumes, and other cosmetic products since the 1970s. The group consists of three major subgroups: nitro musks (e.g., musk ketone and musk xylene), polycyclic musks (e.g., Galaxolide® and Tonalide®), and macrocyclic

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musks (e.g., Exaltone® and Muscone®). The subgroup employed most extensively is by far the polycyclic-musk compounds, followed by nitro musks, which again are used considerably more than the macrocyclic musks.

The presence of synthetic musks in the environment was substantiated by trace chemical analysis approximately a decade after the compounds were introduced in the market. Thus, in the early 1980s synthetic musks were found in fish from the Tama River near Tokyo [1], and a little more than 10 years later the presence of polycyclic musks and nitro musks in the Central European environment [2] was reported. Since then many studies have dealt with the environmental distribution of these chemicals [3, 4], and today such compounds have been proven to be present in practically all environmental compartments [5–7], including human tissue [8, 9], close to densely populated areas. Generally the polycyclic musks are present in the environment in significantly higher concentrations than the nitro musks [10]. Compounds which are going to function as fragrance additives for an extended period of time, have to be unreactive, and it has therefore been taken for granted that the concentration of synthetic musk compounds in environmental samples is adequately determined by analysing the samples with respect to the original musk compounds only. However, in 1999 this assumption was undermined when Franke *et al*. [11] analysed selected environmental samples and identified Galaxolidone, a transformation product from Galaxolide®, and determined its concentration to be comparable to that of Galaxolide® in the same samples. A couple of years later the same transformation product was found in marine fish from Norwegian waters [12], also in this case at concentration levels similar to those found for Galaxolide®.

In addition to Galaxolide®, most environmental samples usually contain Tonalide®, but so far no transformation product from the latter has been detected and identified. Considering the structural similarity between the two polycyclic musk compounds, it is reasonable to assume that Tonalide® has reacted similar to Galaxolide® and afforded one or several transformation products that so far have not been detected. In order to facilitate the detection of such products, we decided to convert Tonalide® to a number of oxidised derivatives that are conceivable metabolites under natural conditions and use these derivatives in search for Tonalide® transformation products in selected environmental samples. As reported here, this appeared to be a successful approach since five such metabolites were detected in samples of both human breast-milk and marine fish.

2. Experimental

2.1 *Chemicals and equipment*

Pure Tonalide® (AHTN) was kindly provided by Fragrance Resources (Clifton, NJ, USA; Lot. 214). All other reactants and reagents were Fluka chemicals, purchased from Sigma-Aldrich (Oslo, Norway), whereas all pure and SupraSolv-quality solvents for synthetic purposes and chemical analysis, respectively, were purchased from VWR-Merck Eurolab (Darmstadt, Germany).

The reaction products were characterised by ^{1}H-NMR, ^{13}C-NMR, and ^{17}O-NMR (Bruker Spectrospin AC200: $^{1}H = 50\,MHz$, $^{13}C = 200\,MHz$; Bruker Spectrospin DRX600: $^{1}H = 600\,MHz$, $^{13}C = 150\,MHz$, $^{17}O = 81.4\,MHz$; Bruker BioSpin,

Karlsruhe, Germany). The solvent and internal reference is given for each spectrum. The signals are characterised as follows: s for singlet, bs for broad singlet, d for doublet, dd for double doublet, t for triplet, q for quartet, and m for multiplet. The chemical shifts are given in ppm, relative to tetramethylsilane (TMS) for ^{1}H and ^{13}C, and to acetone-d_6 for ^{17}O. The coupling constants (J) are given in Hz. Data handling was carried out with MestRe-C V2.3 for 1D spectra and X-WIN NMR V2.6 for 2D spectra.

UV spectra were obtained using a Cary 3 UV-VIS spectrometer (Varian, Palo Alto, CA, USA). Methanol was used as solvent. Data handling was carried out with a CARY Win UV V02.00(25) unit.

IR spectra were recorded on a Nicolet Impact 410 FT-IR spectrometer. The absorptions are characterised as follows: s for strong, m for medium, and w for weak. Data handling was carried out with an OMNIC V1.01 unit.

Mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained on a Fisons VG 7070 E mass spectrometer operated in the EI mode with an ionisation potential of 70 eV.

A high-resolution gas chromatography/flame ionisation detector (HRGC/FID) attached to an HP 5890 gas chromatograph (Agilent, Palo Alto, CA, USA) was used for monitoring the progress of the chemical reactions. The carrier gas was 1 mL s^{-1}, and a DB 5 capillary column (J&W Folsom, CA, USA; characteristics: 0.32 mm inner diameter, 30 m length, 0.25 mm film thickness) was used for chromatographic separation. The following temperature program was used for compound separation: initial temperature 90°C (1 min isotherm), heating with 10°C min^{-1} to 200°C, from 200°C heating with 5°C min^{-1} to 220°C, from 220°C with 30°C min^{-1} to the final temperature of 280°C (10 min isothermal).

A Mega II 8560 gas chromatography (HRGC), with He (5.5 quality, Hydro Gas, Porsgrunn, Norway) as carrier gas and a DB 5-MS capillary column (0.32 mm inner diameter, 30 m length, 0.25 mm film thickness) was used for chromatographic separation. The high-resolution gas chromatograph was coupled to low-resolution mass spectrometer (LRMS: Finnigan MD 800), which was used for structure confirmation and trace analysis (Mega II 8560; Fisons, Milano, Italy, MD 800: Finnigan, San Jose, CA, USA).

All photochemical reactions were performed with a 125 W medium-pressure mercury lamp (model 3010, photochemical reactors, Reading, UK). A water-cooled Pyrex cover with a cut-off at ca. 280 nm wavelength was used as filter.

2.2 *Synthesis of potential transformation products*

The compounds prepared from Tonalide® and the synthetic pathways employed are summarised in figure 1.

2.2.1 3-Acetyl-5,5,7,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (2).

A solution of **1** (0.26 g, 1.01 mmol) and methylene blue (0.16 g, 0.50 mmol) in methanol (170 mL) was irradiated with Pyrex-filtered light ($\lambda > 280$ nm) for 10.5 h in the presence of air. Evaporation of the solvent on a rotary evaporator yielded 0.45 g of a blue residue, from which 0.10 g (37%) of ketoaldehyde **2** was isolated as a yellow solid by column chromatography (silica gel; *n*-hexane : ethyl acetate = 90 : 10), m.p. 99–101°C; UV (λ_{max}, (ε)): 204 (13,000), 230,5 (17,450), 265,5 (7,300); ν_{max} (KBr)/cm^{-1}: 2964(s),

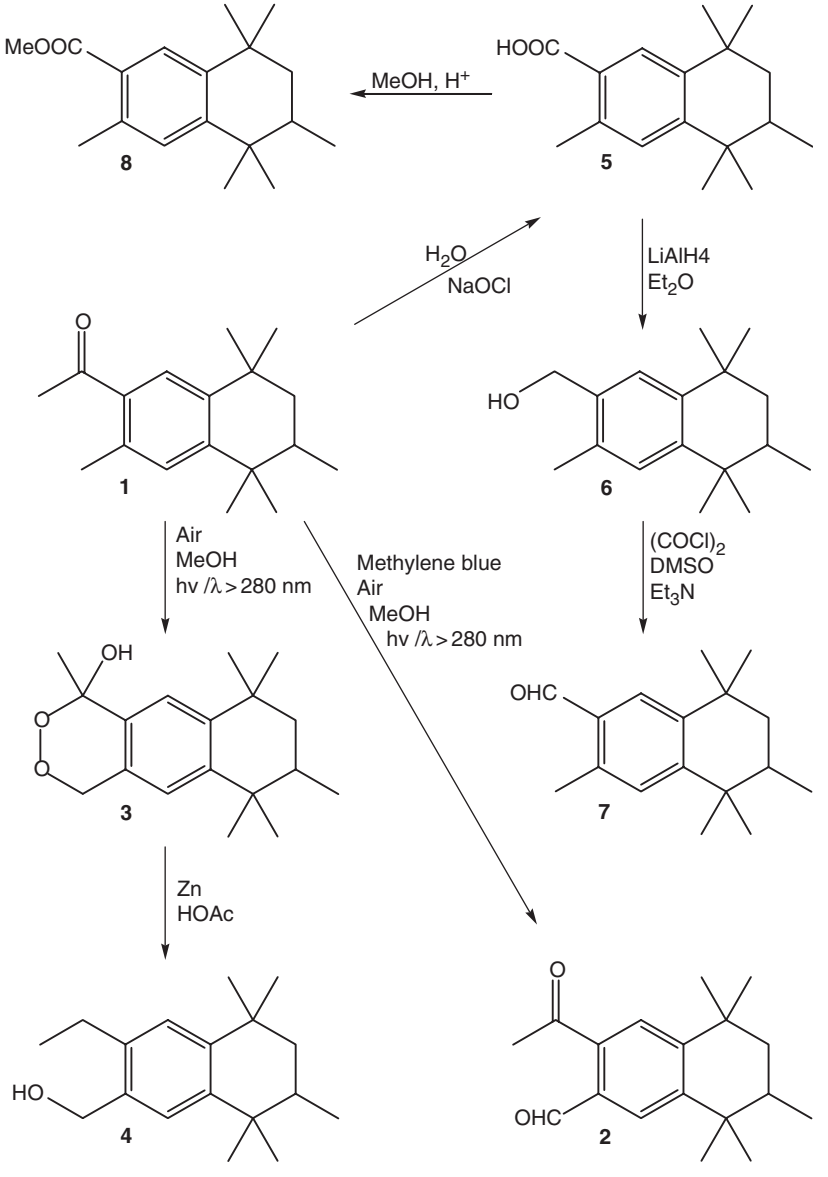

Figure 1. A summary of the reactions used to prepare synthesised standards of Tonalide® derivatives.

2910(s), 2881(s), 1681(s), 1597(m), 1545(m), 1465(m), 1423(w), 1358(m), 1260(s), 1240(m), 1196(w), 1162(m), 1114(m), 1092(m), 992(m), 952(w), 910(m), 790(m), 763(w), 670(w), 649(m), 573(w), 539(w); δ_H (200 MHz; $CDCl_3$; Me_4Si): 10.21 (1H, s), 7.90 (1H, s), 7.64 (1H, s), 2.64 (3H, s), 1.90 (1H, m, $J = 2.6$, 6.8, 12.6), 1.66 (1H, dd, $J = 12.6$, 13.5), 1,46 (1H, dd, $J = 2.6$, 13.5), 1.38 (3H, s), 1.36 (3H, s), 1.31 (3H, s), 1.10 (3H, s), 1.02 (3H, d, $J = 6.8$); δ_C (50 MHz; $CDCl_3$; Me_4Si): 201.1, 192.2, 150.6, 150.4, 137.7, 133.4, 129.0, 127.1, 42.8, 38.1, 34.8, 34.1, 32.0, 31.4, 28.7, 28.1, 24.6, 16.5; MS (m/z, % rel. int): 272 (64, M^+), 257 (10), 229 (100), 215 (8), 197 (11),

187 (36), 173 (29), 155 (8), 141 (11), 128 (16), 115 (13), 91 (8), 77 (6), 57 (18); HRMS Calcd for $C_{18}H_{24}O_2$ 272,177630, found 272,186768.

2.2.2 1,6,6,7,9,9-Hexamethyl-1,4,6,7,8,9-hexahydronaphtho[2,3-d][1,2]dioxin-1-ol (3). A solution of **1** (0.26 g, 1.01 mmol) in methanol (170 mL) was irradiated with Pyrex-filtered light ($\lambda > 280$ nm) for 2 h. Evaporation of the solvent gave 0.30 g of a clear, viscous residue, from which 0.11 g (38%) of peroxide **3** was isolated as a colourless, viscous oil by column chromatography (silica gel; *n*-hexane: ethyl acetate = 90 : 10). UV (λ_{max}, (ε)): 204 (23,250); ν_{max} (NaCl)/cm^{-1}: 3473(m), 3028(m), 2963(s), 2250(w), 1740(w), (1683(w), 1501(m), 1463(s), 1412(s) 1373(s), 246(s), 1143(s), 1075(s), 973(m), 914(s), 877(s), 744(s); δ_H (200 MHz; $CDCl_3$; Me_4Si): 7.40 (1H, s), 7.39 (1H, s), 7.05 (2H, s), 5.38 (1H, d, $J = 15.0$), 5.36 (1H, d, $J = 15.0$), 4.83 (1H, d, $J = 15.0$), 4.80 (1H, d, $J = 15.0$), 3.82 (1H, s), 3.78 (1H, s) 1.88 (2H, m, $J = 2.6$, 6.8, 12.9), 1.65 (2H, dd, $J = 12.9$, 13.5), 1.67 (3H, s), 1.65 (3H, s), 1.39 (2H, dd, $J = 2.6$, 13.5), 1.32 (12H, s), 1.27 (3H, s), 1.26 (3H, s), 1.07 (3H, s), 1.05 (3H, s), 1.00 (3H, d, $J = 6.8$); δ_C (50 MHz; $CDCl_3$; Me_4Si): 146.2, 146.1, 144.4 (2× C), 133.0, 132.9, 128.9, 128.8, 123.6 (2× C), 122.2 (2× C), 99.4 (2× C), 71.8, 71.7, 43.2 (2× C), 37.6 (2× C), 34.2 (4× C), 32.6, 32.2, 32.0, 31.8, 28.5, 28.4, 25.0, 24.9, 23.6, 23.5, 16.7, 16.6; δ_O (81.4 MHz; acetone-d_6; –): 75, 235, 290; MS (*m*/*z*, % rel. int): 290 (1, M^+), 287 (7), 273 (100), 257 (45), 343 (58), 229 (58), 213 (17), 199 (18), 187 (25), 173 (18), 157 (6), 141 (10), 128 (14), 115 (10), 91 (6), 84 (18), 57 (21).

2.2.3 (3-Ethyl-5,5,7,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene-2-yl)methanol (4). To a stirred solution of peroxide **3** (0.08 g, 0.28 mmol) in a mixture of glacial acetic acid (10 mL) and water (2 mL) was added activated zinc powder (0.50 g, 7.65 mmol) as described by Bayley *et al.* [13]. After 1.5 h the product mixture was extracted three times with dichloromethane (CH_2Cl_2). The extracts were combined, dried with $MgSO_4$, and filtered. Evaporation of the solvent under vacuum gave 0.08 g of a yellow, viscous residue, from which 0.01 g (14%) of alcohol **4** was isolated pure as a colourless, viscous oil by column chromatography (silica gel; gradient elution with *n*-hexane/ethyl acetate mixtures). ν_{max} (NaCl)/cm^{-1}: 3346(m), 2962(s), 2952(s), 2872(s), 1754(m), 1499(m), 1456(s), 1396(m), 1365(w), 1261(m), 1222(w), 1197(w), 1088(m), 1058(m), 1021(m), 894(m), 802(m); δ_H (200 MHz; $CDCl_3$; Me_4Si): 7.33 (1H, s), 7.15 (1H, s), 4.69 (2H, s), 2.69 (2H, q, $J = 7.6$), 1.87 (1H, m, $J = 2.6$, 6.8, 12.9), 1.64 (1H, dd, $J = 12.9$, 13.2), 1.60 (1H, b s), 1.38 (1H, dd, $J = 2.6$, 13.2), 1.33 (3H, s), 1.30 (3H, s), 1.26 (3H, s), 1.25 (3H, t, $J = 7.6$), 1.07 (3H, s), 0.98 (3H, d, $J = 6.8$); δ_C (50 MHz; $CDCl_3$; Me_4Si): 144.4, 143.6, 139.0, 135.3, 126.8, 126.4, 63.3, 43.5, 37.4, 34.5, 34.1, 32.2, 31.9, 28.5, 24.9, 24.8, 16.7, 15.2; MS (*m*/*z*, % rel. int): 260 (15, M^+), 245 (90), 185 (14), 173 (32), 159 (10), 57 (100).

2.2.4 3,5,5,6,8,8-Hexamethyl-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid (5). A mixture of **1** (2.58 g, 9.98 mmol) and 10% aqueous NaOCl (150 mL) was stirred at 70°C. After 17 h the mixture was cooled to room temperature, acidified with 6 M hydrochloric acid, and extracted three times with dichloromethane (CH_2Cl_2). The extracts were combined, dried with $MgSO_4$, and filtered. Evaporation of the solvent under vacuum gave 2.63 g of a white, solid residue, from which 2.01 g (77%)

of carboxylic acid **5** was isolated as white crystals upon recrystallisation from diethyl ether, m.p. 214°C; UV (λ_{max}, (ε)): 212.5 (17,700), 241.5 (10,200), 285.5 (1,300); ν_{max} (KBr)/cm^{-1}: 2964(s), 2923(s), 2648(m), 2553(m), 1685(s), 1608(m), 1550(m), 1501(w), 1460(m), 1410(m), 1385(m), 1374(m), 1312(s), 1264(s), 1196(m), 1123(s), 1038(w), 943(m), 926(m), 885(m), 807(w), 707(m), 651(w), 532(m); δ_H (600 MHz; $CDCl_3$; Me_4Si): 12.60 (1H, b s), 8.05 (1H, s), 7.23 (1H, s), 2.61 (3H, s), 1.88 (1H, m, $J = 2.7$, 7.0, 12.9), 1.64 (1H, dd, $J = 12.9$, 13.4), 1.40 (1H, dd, $J = 2.7$, 13.4), 1.34 (3H, s), 1.33 (3H, s), 1.27 (3H, s), 1.08 (3H, s), 1.00 (3H, d, $J = 7.0$); δ_C (150 MHz; $CDCl_3$; Me_4Si): 173.4, 151.6, 142.5, 137.8, 130.4, 130.3, 125.5, 43.4, 37.9, 34.3, 33.9, 32.2, 31.8, 28.2, 24.6, 21.9, 16.7; MS (*m*/*z*, % rel. int): 260 (17, M^+), 245 (100), 203 (13), 189 (12), 159 (35), 145 (13), 128 (14), 115 (12), 105 (8), 91 (6), 57 (12); HRMS Calcd for $C_{17}H_{24}O$ 260,177630, found 260,174164.

2.2.5 (3,5,5,6,8,8-Hexamethyl-5,6,7,8-tetrahydronaphthalene-2-yl)methanol (6). A solution of carboxylic acid **5** (1.04 g, 3,99 mmol) in dry diethyl ether (80 mL) was added during 1 h to a stirred suspension of lithium aluminium hydride (LAH) (0.54 g, 14.22 mmol) in dry diethyl ether (40 mL) kept under nitrogen at 50°C. After 0.5 h the mixture was cooled with an ice/water bath and hydrolysed with water (120 mL) followed by 6 M hydrochloric acid (90 mL) (to dissolve precipitated $Al(OH)_3$). The hydrolysate was extracted three times with dichloromethane (CH_2Cl_2), and the combined extracts were dried with $MgSO_4$ and filtered. Evaporation of the solvent under vacuum gave 0.97 g (98%) of alcohol **6** as a pure, white solid. m.p. 104°C; UV (λ_{max}, (ε)): 208 (12,050), 216.5 (10,450), 268.5 (550), 276.5 (550); ν_{max} (KBr)/cm^{-1}: 3312(s), 2960(s), 1499(m), 1462(s), 1400(m), 1362(m), 1303(w), 1282(w), 1265(w), 1219(m), 1054(s), 1024(s), 999(m), 893(m); δ_H (200 MHz; $CDCl_3$; Me_4Si): 7.26 (1H, s), 7.15 (1H, s), 4.61 (2H, s), 2.61 (3H, s), 2.01 (1H, b s), 1.88 (1H, m, $J = 2.6$, 6.8, 12.6), 1.62 (1H, dd, $J = 12.6$, 13.2), 1,36 (1H, dd, $J = 2.6$, 13.2), 1.31 (3H, s), 1.30 (3H, s) 1.25 (3H, s), 1.05 (3H, s), 0.98 (3H, d, $J = 6.8$); δ_C (50 MHz; $CDCl_3$; Me_4Si): 145.4, 142.2, 135.7, 133.0, 128.7, 125.7, 63.4, 43.5, 37.3, 34.4, 33.9, 32.2, 31.9, 28.4, 24.8, 18.3, 16.7; MS (*m*/*z*, % rel. int): 246 (21, M^+), 231 (100), 215 (17), 201 (7), 171 (24), 159 (43), 145 (13), 129 (10), 115 (8), 105 (9), 91 (9), 57 (15); HRMS Calcd for $C_{17}H_{26}O$ 246,190366, found 246,190254.

2.2.6 3,5,5,6,8,8-Hexamethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (7). A mixture of dry dimethylsulfoxide (DMSO) (0.40 mL, 5.61 mmol) and dry dichloromethane (CH_2Cl_2) (1 mL) was first added carefully during 5 min to a stirred solution of oxallyl chloride (0.20 mL, 2.29 mmol) in dry dichloromethane (CH_2Cl_2) (5 mL) kept under nitrogen at −60°C. Fifteen minutes later a solution of alcohol **6** (0.50 g, 2.03 mmol) in dry dichloromethane (CH_2Cl_2) (2 mL) was added carefully during 5 min. After stirring, still at −60°C, for 40 min triethylamine (1.5 mL, 10.79 mmol) was added dropwise with care during 5 min. The cooling bath was removed, and when the solution had reached room temperature, hydrolysis with water (7 mL) and acidification with 6 M hydrochloric acid were carried out. The hydrolysate was extracted three times with dichloromethane (CH_2Cl_2). The extracts were combined, dried with $MgSO_4$, and filtered. Evaporation of the solvent under vacuum gave 0.50 g of a yellow solid, from which 0.43 g (87%) of aldehyde **7** was isolated pure

as a white solid by column chromatography (silica gel; *n*-hexane : ethyl acetate = 90 : 10). The compound turned yellowish on standing. m.p. 82°C; UV (λ_{max}, (ε)): 217 (17,600), 262 (13,800), 302 (2,000); ν_{max} (KBr)/cm^{-1}: 2964(s), 2924(s), 2755(w), 2700(w), 1695(s), 1604(m), 1547(m), 1497(w), 1456(m), 1387(m), 1366(m), 1214(s), 1147(m), 1112(m), 1028(w), 1003(m), 889(m), 805(m), 763(w), 577(w); δ_H (200 MHz; $CDCl_3$; Me_4Si): 10.19 (1H, s), 7.74 (1H, s), 7.22 (1H, s), 2.61 (3H, s), 1.89 (1H, m, $J = 2.6$, 6.5, 12.6), 1.63 (1H, dd, $J = 12.6$, 13.5), 1.41 (1H, dd, $J = 2.6$, 13.5), 1.34 (6H, s), 1.27 (3H, s), 1.08 (3H, s), 1.00 (3H, d, $J = 6.5$); δ_C (50 MHz; $CDCl_3$; Me_4Si): 191.6, 151.5, 142.0, 135.9, 130.9, 130.0, 129.4, 42.2, 37.1, 33.3, 33.0, 31.3, 30.8, 27.2, 23.6, 18.3, 15.7; MS (*m*/*z*, % rel. int): 244 (34, M^+), 229 (100), 187 (24), 173 (24), 159 (33), 145 (10), 128 (9), 115 (6), 105 (6), 57 (11); HRMS Calcd for $C_{17}H_{24}O$ 244,182716, found 244,181816.

2.2.7 Methyl 3,5,5,6,8,8-hexamethyl-5,6,7,8-tetrahydronaphthalene-2-carboxylate (8). A solution of carboxylic acid **5** (0.07 g, 0.27 mmol) in methanol (5 mL), containing three drops of concentrated sulfuric acid, was stirred at 75°C. After 13 h the methanol was evaporated. The residue obtained was dissolved in dichloromethane (CH_2Cl_2), and the resulting solution was washed three times with a dilute aqueous solution of sodium hydroxide. The organic solution was dried with $MgSO_4$ and filtered. Evaporation of the solvent under vacuum gave 0.08 g (100%) of ester **8** as a viscous oil which crystallised to a yellowish solid after a short while, m.p. 67°C; UV (λ_{max}, (ε)): 213 (18,700), 242.5 (12,800), 285 (1,700); ν_{max} (NaCl)/cm^{-1}: 3030(m), 2963(s), 1716(s), 1616(m), 1558(m), 1505(m), 1456(s), 1386(m), 1381(m), 1300(s), 1263(s), 1197(s), 1144(m), 1102(s), 1041(m), 1025(m), 992(m), 883(m), 820(m), 787(m), 725(w), 561(w); δ_H (200 MHz; $CDCl_3$; Me_4Si): 7.88 (1H, s), 7.20 (1H, s), 3.87 (3H, s), 2.55 (3H, s), 1.87 (1H, m, $J = 2.6$, 6.8, 12.9), 1.62 (1H, dd, $J = 12.9$, 13.2), 1,36 (1H, dd, $J = 2.6$, 13.2), 1.32 (6H, s), 1.26 (3H, s), 1.06 (3H, s), 0.97 (3H, d, $J = 6.8$); δ_C (50 MHz; $CDCl_3$; Me_4Si): 168.0, 150.4, 142.2, 136.7, 130.1, 129.0, 126.6, 51.5, 43.3, 37.7, 34.2, 33.9, 23.2, 31.8, 28.2, 24.6, 21.5, 16.7; MS (*m*/*z*, % rel. int): 274 (20, M^+), 259 (100), 243 (9), 217 (16), 203 (14), 185 (11), 171 (5), 157 (7), 143 (6), 128 (5), 57 (6); HRMS Calcd for $C_{18}H_{26}O_2$ 274,193280, found 274,191635.

3. Sample preparation

Extracts from three human breast-milk samples and four marine-fish samples (one saithe filet, *Pollachius virens*, one thornback ray filet, *Raja clavata*, and two haddock filet samples, *Melanogrammus aeglefinus*) were analysed with respect to compounds **2**–**8** derived from AHTN. The milk samples were provided by the Bodø General Hospital (Nordland county, Norway), and the saithe sample was caught in the Tromsø harbour (Troms county, Norway), whereas the other fish samples were caught in the Trondheim harbour (Trøndelag county, Norway).

The preparation of the samples employed has been described previously; the method employed has been adapted for the analyses of the samples with respect to Tonalide® (AHTN), Traseolide® (ATII), Galaxolide® (HHCB), and Galaxolide® derivatives [12].

Table 1. The average concentration of Tonalide® derivatives **2**, **4**, **6**, **7**, and **8** as determined by SIM analysis.[a,b]

	Average concentration (pg g^{-1} wet weight)				
Sample	**2**	**4**	**6**	**7**	**8**
Breast milk	18	9	n.d.	n.d.	9
Breast milk	17	n.d.	n.d.	n.d.	8
Breast milk	24	7	n.d.	n.d.	n.d.
Haddock	n.d.	9	n.d.	8	n.d.
Haddock	10	n.d.	n.d.	n.d.	n.d.
Saithe	9	n.d.	n.d.	n.d.	n.d.
Thornback ray	n.d.	n.d.	18	n.d.	n.d.

[a] The analytical method has not been calibrated; the concentrations are therefore indicative only; [b] n.d. = not detected.

4. Quality control and trace analysis

4.1 *Restrictions and strategies*

Unfortunately, the extracts prepared from the seven biological samples collected were not prepared by methods completely validated for the quantitative trace analysis of compounds **1**–**8**. Although their response factors were calculated base upon a first linearity experiment using standard solutions of four different concentrations (25, 50, 100 and 125 pg μL^{-1}), a complete method validation was not available at the time of the experiment. Thus, the concentrations reported for the compounds detected (table 1) should therefore solely be regarded as semi-quantitative estimates and as a first indication of the presence of AHTN transformation products in the environmental samples. Preliminary investigation of extraction efficiency and method recovery indicates that the properties of the synthesised standards **2**–**8** in these respects are similar to those of the parent compound AHTN. A thorough evaluation of the methods used to quantify these transformation products in the environmental samples is intended be published in due course.

4.1.1 Standard preparation. Standard solutions of each of the naphthalene derivatives **1**–**8** in isooctane were made, and the response factor, retention time, and linearity range for each compound were determined by the HRGC/LRMS method. The following temperature program was used for gas chromatographic separation: 90°C (6 min. isothermal), heating with 6°C min^{-1} to the final temperature of 280°C (6 min. isothermal). For each compound the GC retention time and the two most abundant ions used to perform selected-ion-monitoring (SIM) MS analyses are listed in table 2.

All extracts were analysed by SIM fragmentogram in electron impact (EI) mode of the two most abundant ions for each of the synthesised standards (table 2). A deviation of +/− 5% from the average concentration value of each of the ions was achieved. The concentration of the compounds detected in each extract is listed in table 1.

Two laboratory blanks and two method blanks were analysed in parallel to detect any laboratory contamination. No contamination was found in any of these blanks.

Table 2. Retention times and the two characteristic ions used to perform SIM analysis of each of the Tonalide® derivatives **2**, **4**, **6**, **7**, and **8**.

Tonalide® derivative	Retention times (min)	Ions for SIM analysis (m/z)
2	13.0	229 and 272
4	11.3	245 and 260
6	11.1	231 and 246
7	10.8	229 and 244
8	11.3	259 and 274

5. Results and discussions

Although several polycyclic musk compounds have been detected in environmental samples over the years, only one transformation product, a lactone derived from Galaxolide® (HHCB), had been reported when this investigation was started [11, 12]. The detection of several compounds derived from Tonalide® is therefore significant and scientifically interesting.

The results presented here are based on the assumption that analytical methods, which are fully validated for structurally related compounds, can be used to obtain a first, semi-quantitative estimate of environmental pollutants, for which no validated analytical method exists. Such an approach should be interesting for authorities and environmental agencies, which often need to know whether new environmental pollutants are present or not.

Five of the seven compounds synthesised were detected by HRGC/LRMS analyses of the extracts. The most abundant derivative was 3-acetyl-5,5,7,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (**2**), which was proved to be present in five of the seven samples. The compound can be envisaged to result from benzylic oxidation of the Tonalide® methyl group, a process which is well-known to take place when methylated benzenes undergo photooxidation [14]. The concentrations of **2**, which were between 9 and 24 pg g^{-1} wet weight, were significantly higher in the milk samples than in the fish samples (table 1).

The second most abundant Tonalide® derivative was (3-ethyl-5,5,7,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene-2-yl)methanol (**4**), which was present in three extracts (7–9 pg g^{-1} wet weight), two of the milk samples and one of the haddock filets. The formation of **4** involves reduction as well as oxidation of **1**, with reduction as the overall result.

(3,5,5,6,8,8-Hexamethyl-5,6,7,8-tetrahydronaphthalene-2-yl)methanol (**6**) was proved to be present in one fish sample only, the thornback ray filet (18 pg g^{-1} wet weight, table 1). Similarly, 3,5,5,6,8,8-hexamethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (**7**) was only detected in one of the Haddock filet samples, at a relatively low concentration (8 pg g^{-1} wet weight). The significance of these observations requires further studies.

The fifth Tonalide® derivative detected was methyl 3,5,5,6,8,8-hexamethyl-5,6,7,8-tetrahydronaphthalene-2-carboxylate (**8**), the corresponding methyl ester of acid **5** (figure 1), which conceivably is formed by methylation during work-up. This ester was found at low levels (8–9 pg g^{-1} wet weight) in two breast-milk samples.

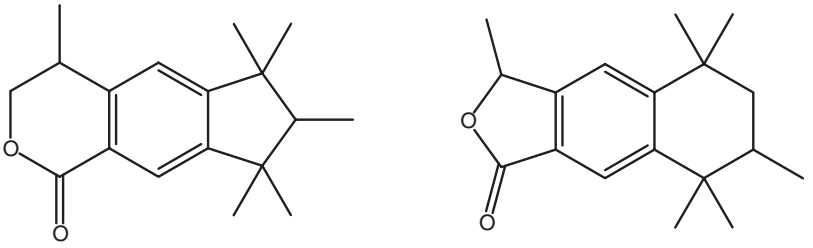

Figure 2. The lactones of Galaxolide® and Tonalide®.

Based on literature information about the main transformation product of Galaxolide®, the HHCB-lactone, it was expected that the analogous product from Tonalide®, the AHTN-lactone (**9**), would appear to be one of the main transformation products in environmental degradation of the latter synthetic musk compound (figure 2). However, analyses of our samples of human milk and marine fishes did not show the presence of AHTN-lactone (**9**). Since the trace-analytical method employed is tested and proved valid for the HHCB-lactone [12] and the chemical structures are relatively similar, it can be assumed that this method is also applicable for the quantitative determination for AHTN-lactone.

On the other hand, indications were found that five of the seven isolated transformation products of Tonalide® are present in the environment. Compound **2** was identified in five of the seven samples with the highest levels in the three human breast-milk samples (17–24 pg g^{-1} wet weight). It is noteworthy that in all breast-milk samples, the levels of the parent compound Tonalide® were very low, close to or below the detection limit, whereas transformation product **2** was found in measurable amounts. Thus, these first results indicate the environmental relevance of this newly identified Tonalide® transformation product. Compound **4** can also be considered as identified xenobiotic in the environment found in two breast-milk and one haddock filet sample in concentrations slightly above the detection limit. This compound seems to be evenly distributed between human breast-milk and marine fish samples (7–9 pg g^{-1} wet weight). However, the detected values are close to the detection limits.

Except for compounds **2** and **4**, the final confirmation of the environmental presence needs further clarification for all newly isolated transformation products. However, the presence of compound **2** in the environment should be considered as confirmed. It should be stressed again, that all results presented here must be considered as qualitative due to incomplete method evaluation for the identified compounds, although the method is proved valid for the parent compound Tonalide®. Therefore, the suitability of the applied analytical method needs thorough documentation and evaluation for all compounds before quality controlled quantitative results can be presented and discussed. Thus, in the ongoing research effort, extraction method, clean-up procedure (gel permeation chromatography, silica gel), and final fractionation are evaluated concerning the applicability for the new transformation products based on valid quality control measures in analytical chemistry. Based on the outcome of this study, a comprehensive number of environmental samples will be analysed with regard to the new contaminants identified.

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