Environmental and human health concerns relating to synthetic musk compounds

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Executive summary

Synthetic musks are man-made aromatic compounds that are used in place of more expensive natural musks. They are added to many everyday products, including laundry detergents, air fresheners, hand creams, soaps and perfumes

The term synthetic musks encompasses three broad chemical groups, namely nitromusks, polycyclic musks and macrocyclic musks. Due to toxicological concerns, nitromusk production has been in decline in Europe for a number of years. Only two nitromusks are of importance today: musk xylene (MX) and musk ketone (MK). These, along with two polycyclic musks, galaxolide (HHCB) and tonalide (AHTN) account for 95% of the European market for synthetic musks.

Synthetic musks are environmentally persistent chemicals and, as a consequence of this and their extensive use in products, have become widely distributed in the environment, especially in aquatic and marine systems but also in the atmosphere and inside buildings. A study commissioned by Greenpeace Netherlands of chemicals in rainwater within the Netherlands found synthetic musk compounds in virtually all rainwater samples. While HHCB was found to be distributed fairly evenly, there was a clear peak in levels of AHTN in the centre of the country, coinciding with the location of a synthetic musk production facility. Significantly, the nitro musk musk ambrette (MA), which has been banned in the EU since 1995, was found at 34% of the rainwater collection points, suggesting long-term environmental persistence.

Synthetic musks can concentrate in living tissues; indeed, musks used in perfumes have also been found contaminating human blood and breast milk. There is increasing evidence emerging that some nitromusks and polycyclic musks, including those commonly used in perfumes, may be capable (either as parent compounds or as metabolites) of interfering with hormone communication systems in fish, amphibians and mammals, and may exacerbate the effects of exposure to other toxic chemicals.

Although the oestrogenic activity exhibited by HHCB and AHTN in mammals is relatively weak, anti-oestrogenic effects have been observed for the same compounds at concentrations more than 100 times lower. Statistical associations have been reported between MX and MK levels in the blood and the occurrence of certain gynaecological conditions in women, though a causal relationship has not been established.

1. Introduction

Musks are considered essential compounds by the fragrance industry and are widely used in cosmetics, detergents, fabric softeners, cleaning products and other household products (OSPAR 2004). By far the bulk of musk compounds in use today are synthetic musks. Natural musk is secreted by the musk deer, a rare mammal of the Asiatic highlands. The first synthetic musk, a nitro musk, was discovered accidentally by a chemist researching explosives in the late 19th century.

For many years nitro musks dominated the synthetic musk market. However, due to emerging toxicological concerns nitro musk production has been in decline in Europe for a number of years. Only two nitro musks are of importance today; musk xylene (MX) and musk ketone (MK). These, along with two polycyclic musks, HHCB (Galaxolide) and AHTN (Tonalide), account for 95% of European market (OSPAR 2004). Regulatory pressure and mounting environmental concerns are increasing pressure to stop using both musk xylene and musk ketone (OSPAR 2004). At the same time, as a result of emerging concerns relating to polycyclic musks, a new class of musks, macrocyclics, are proposed as substitutes.

This document provides an overview of the literature available on all three classes. There is a lack of research on the environmental fate and risk posed by macrocyclic musks and so the information presented on these compounds is brief.

2. Physiochemical Data

Both nitro musks and polycyclic musks are poorly water soluble and exhibit relatively high octanol/water partition coefficients.

Compound	CAS	Solubility (mg/l)	Log K_{ow}	Log K_{oc}	Ref.
Musk xylene	81-15-2	0.15	4.9		EC 2003a
Musk ketone	81-14-1	0.46	4.3		EC 2003b
HHCB	1222-05-5	1.75	5.9	5.86	Balk & Ford 1999a
AHTN	1506-02-1	1.25	5.7	5.80	

3. Usage

Estimates suggest that in the year 2000, MX, MK, HHCB and AHTN made up the bulk of European consumption, with the two polycyclic musks accounting for over 90% of consumption. No accurate data on macrocyclic musk usage was available. In 1997 of the 8,000 tonnes of synthetic musk produced world wide, it was estimated that macrocyclic musks accounted for only around 5% (cited in Bitsch *et al* 2002). Since then this may have increased due to the significant reduction in production of nitro musks (OSPAR 2004). Several recent studies have documented the presence of musks in consumer goods, especially perfumes and consumer care products.

4. Fate in Waste Water Treatment Plants

It can be expected that a major component of synthetic musk releases to the environment arises via waste water as a result of their use in consumer products (Balk and Ford 1999 a). Due to limited water solubility and strong adsorptive properties, the compounds are associated with particulate matter. Nitro musks are typically present in waste water treatment plant (WWTP) influent at concentrations from 0.1-1ugl^{-1} (Kanda 2003, Simonich 2002). Levels are lower for plants primarily treating non-domestic waste water. Influent concentrations of AHTN and HHCB are typically an order of magnitude greater (1-15 ugl^{-1}), reflecting greater prevalence in products (Kanda *et al* 2003, Simonich *et al* 2002, OSPAR 2004). The less used musks, including the nitro musks, musk ambrette, musk moskene and the polycyclics ADBI, AHMI and AITI if detected at all, are always at much lower concentrations (OSPAR 2004).

Concentrations of MX and MK in WWTP effluent are generally of an order of magnitude lower than those in influent (Kanda *et al* 2003, Simonich *et al* 2002, OSPAR 2004). Biological reduction of nitro groups to amino metabolites has been shown to be a major loss pathway in both sludge microbes and higher organisms. Gattermann *et al* (1998) found the metabolites 4-amino-MX and 2-amino-MX at concentrations equal to and 3.5 times greater than the parent musks respectively in effluent. Concentrations of the only primary metabolite of MK, 2-amino-MK was determined at levels 40 times greater than those of the parent. This indicates the importance of a rigorous study of environmental pathways when determining the impact of substances. The balance of musk compounds entering the plant is likely to be absorbed to sludge; Herren and Berset (2000) report the presence of nitro musks in sludge and detected MX and MK amino derivatives at concentrations greater than the parent compounds. Very little is know about the ultimate fate of this substantive fraction.

Concentrations of HHCB and AHTN in the liquid phase can be reduced by over 80% during waste water treatment (Balk and Ford 1999 a). Metabolic pathways are even less well understood than for the nitro musks (Eschke 2004). The lactone derivative of HHCB has been reported as the major metabolite in sewage sludge (Balk and Ford 1999 a) and has been found in WWTP effluent at concentrations one order of magnitude lower than those of HHCB (Biselli *et al* 2004) . Due to high K_{oc} values, it is assumed that a large amount of both compounds are adsorbed to sludge. This is supported by evidence that the difference in influent-effluent concentrations is relatively independent of the type of sewage processing used.

5. Environmental prevalence

Fromme *et al* (2004) detected AHTN and HHCB in kindergarten air with median concentrations of 44 and 101ngm^{-3} respectively (sample size:74). The compounds were also found in apartment air and dust samples, MX and MK have also been detected in indoor air (Kallenborn & Gatermann 2004). Peters (2003) found HHCB in all of 50 rainwater samples collected predominantly in the Netherlands, and AHTN in 80%. Nitro musks were detected in approximately 30% of the samples with elevated levels corresponding to location of know production facilities.

The prevalence of synthetic musks in surface waters has been well documented (Eschke 2004). Bester *et al* (1998) detected AHTN and HHCB in the North Sea (German Bight).

Relative concentrations generally reflect the consumption levels already reported with respect to nitro and polycyclic mucks.

Buerge *et al* (2003) have demonstrated that, in summer, photochemical degradation of AHTN represents a major elimination pathway from a surface waters of a lake; this was found to not be the case for HHCB.

Data on the environmental fate of musk metabolites could not be found.

MK and MX have been evaluated to be environmentally persistent substances, whilst AHTN and HHCB are potentially persistent (OSPAR 2004).

6. Aquatic Biota Concentrations and Accumulation

MX, MK, HHCB and AHTN have been detected in both fresh water and marine biota. Typical concentrations of HHCB and AHTN in Europe range from 0.1-5 mg kg^{-1} lw. In freshwater biota, concentrations of the two polycyclic musks tend to be one to two orders of magnitude higher than those of the nitro musks. Marine mussels, fish and shrimp contain musk levels an order of magnitude lower than their fresh water equivalents (Leonards & Boer 2004). Trends indicate diminished concentrations as distance from WWTP increases. A comparative study by Gattermann *et al* (1999) found that, in Canada, musk ketone exceeded levels of polycyclic musks; this difference between continents reflects usage patterns.

Concentrations of nitro musk transformation products have been reported for freshwater fish (Rimkus *et al* 1999 a). These show species dependant accumulation, and suggest species dependant biotransformation.

A literature review by Leonards & Boer (2004) reported concentrations of polycyclic musks per kg of lipid in eels to be lower than in other fish. This is taken by the authors to suggest that eels have a greater capacity to metabolise polycyclic musks. However, this conclusion assumes that a steady state environment exists. Furthermore, given the far higher lipid content of eels (as a percentage of body weight), on the basis of evidence presented, this conclusion must remain open to question. The authors also suggest that high levels found in pike-perch relative to eels and some other fish indicates biomagnification, though again conclusion must be considered as tentative.

Despite exhibiting a high lipophilicity and bioaccumulation potential there is evidence that synthetic musk concentrations in biota are largely dependant upon immediate environmental concentrations to which the organisms are exposed. van Dijk (cited in Rimkus 1999 b) found that both HHCB and AHTN were rapidly converted to polar metabolites and excreted from bluegill sunfish (*Lepomis macrochirus*). This finding has been supported by other studies (also cited in Rimkus 1999). To date the identity of these polar metabolites has not been elucidated and so no assessment of the risk they pose is possible. Biselli *et al* (2004) present conclusive evidence of species-dependant primary metabolisation of HHCB to HHCB-lactone. Although there is rather limited evidence for biomagnification (through the food chain), the ubiquitous presence of synthetic musks in the aquatic environment of Europe could result in continuous background exposures. The long-term consequences of this are not known.

The bioconcentration factor (BCF) of musk xylene in fish has been determined in a number of studies. These present conflicting results possibly as a consequence of the different methodologies used. At European level (EC 2003 a), and employing a 'weight of evidence' approach, a value of 4100 was considered to be suitable for risk assessment purposes. Musk xylene can therefore be considered a bioaccumulative chemical in strict accordance to EC criteria.

The BCF of musk ketone in fish is subject to similar uncertainties. EC (2003 b) suggests a value of 1380 for conducting risk assessments a value which falls belo the criteria for listing as a bioaccumulative chemical. Though it must be noted that in this case as smaller body of evidence was considered.

BCF values for HHCB and AHTN tend to be lower. Reported values are less than 2000 - typically around 500 (OSPAR 2004) indicating that neither compound would be classified as bioaccumulative according to EU criteria, although both are clearly able to accumulate in biological tissues.

Few data are available on synthetic musk concentrations in higher organisms in the aquatic environment. Leonards & Boer (2004) found HHCB and AHTN in the livers of Danish otters. The high concentrations (104 and 95 mg kg^{-1} lw respectively) are taken by the authors as evidence of biomagnification, suggesting that it may be necessary to revisit the EU's current evaluation process.

7. Ecotoxicity in the Aquatic Environment

The aquatic biota toxicity of musk xylene and musk ketone has been well studied. Acute toxicity is in most cases observed at concentrations at or above the substances' water solubilities (which as noted above are relatively low) (EC 2003 a). The acute toxicity of metabolites of musk xylene to *Daphnia magna* have been reported to be similarly high (Behechti *et al* 1998 & Giddings *et al* 2000). The EC risk assessment exercise for musk xylene used evidence of reproductive toxicity in *Daphnia magna* to determine a Predicted No Effect Concentration (PNEC) of $1.1 ugl^{-1}$. The risk assessment exercise conducted for musk ketone derived a PNEC of 6.3 ugl^{-1} from fish studies. These values indicate that both substances can be considered as toxic under proposed REACH criteria.

Balk and Ford (1999 b) determined Predicted No Effect Concentrations (PNEC) of 3.5 ugl^{-1} (AHTN) and 6.8 ugl^{-1} (HHCB) for aquatic organisms. These values are in accordance with data published more recently and would give rise to toxic classification under the proposed REACH criteria.

Species dependant developmental toxicity has been observed for both nitro and polycyclic musks (Dietrich and Hitzfeld 2004). Results appear to be very study specific. For example the findings of Chou and Dietrich (1999 a) indicate no developmental effects in zebra fish *Danio rerio* in 96h tests with MX and MK. Yet Carlsson and Norrgren (2004) report specific effects on the same species at relatively low concentrations (MK:10 ugl^{-1} amd MX 33 ugl^{-1}). Inhibition of larval development in the crustacean *Acartia tonsa* has been reported at similar concentrations with HHCB, AHTN and MK (Wollenberger *et al* 2004)

Estrogenic effects of polycyclic musks have been reported in *in vitro* competitive binding assays with South African clawed frogs *Xenopus laevis* and rainbow trout *Oncorhynchus mykiss* (Dietrich and Hitzfeld 2004). Interestingly, neither MK nor MX exhibited similar activity, yet all 3 major metabolites (2-amino-MK, 4-amino-MX and 2-amino-MX) did show competitive binding (Chou and Dietrich 1999 b). It must be noted that observed estrogenic activity was weak relative to oestradiol. Again species-specific effects are apparent as AHTN and HHCB showed no activity in carp (Dietrich and Hitzfeld 2004). Schreurs *et al* (2004) reported antiestrogenic effects in zebrafish with HHCB and AHTN in both *in vivo* and *in vitro* studies. This compounds the evidence for very specific organism responses to synthetic musks.

Smital *et al* (2004) recently demonstrated that HHCB, AHTN and musk xylene (musk ketone not tested) can inhibit the multixenobiotic defences of cells taken from mussel gills. The musks inhibited the activity of proteins that usually prevent uptake of xenobiotic agents. As well as illustrating a potential toxicological issue associated with musks, this work shows how standard toxicity tests do not provide a comprehensive picture of the effects a compound may have upon release into the environment.

8. Mammalian Toxicity

Information on hazards to mammals (including humans) is limited. It is known that MX and MK can penetrate the skin (Hawkins *et al* cited in Brunn *et al* 2004). While skin penetration rates measured for the polycyclics AHTN and HHCB appear to be much lower, the presence of these compounds in human fat is evidence that these chemicals can nevertheless readily enter the body (Rimkus & Wolf 1996). Levels of polycyclics in breast milk tend to be higher than concentrations of MX and MK (Rimkus & Wolf 1996), probably reflecting the current balance of use of these different chemicals within Europe.

Whereas acute toxicity of synthetic musks to mammals seems to be relatively low, insufficient data are available to evaluate the hazards of long-term, low level exposure. This is especially true of the polycyclics, including AHTN and HHCB, and their metabolites. Some effects on reproduction and foetal development have been observed in rats, though so far only at levels far higher than ambient exposure levels from consumer products and environmental contamination (Christian *et al* 1999). Evidence presented below reflects the current state of research

Overall it must be concluded that evidence is tentative or incomplete, demonstrating the need for a precautionary approach at least until is possible to demonstrate, with substantive evidence, that these compounds do not pose a risk to humans or the environment as a whole.

Seinen *et al* (1999) investigated the effects of AHTN and HHCB in ERa- and ERb-dependent gene transcription assays with Human Embryonal Kidney 293 (HEK293) cells in order to estimate the compound's' estrogenic activity. Both AHTN and HHCB were found to induce a slight but clearly dose-dependent stimulation of transcriptional activity in the transiently ERa transfected HEK293 cells. However it was concluded that these compounds have very weak estrogenic potency, probably too weak to induce estrogenic effects in wildlife species or humans at the (then) current levels of exposure. Schreurs *et al* (2002) similarly report that AHTN and HHCB act as selective estrogen receptor modulators (SERMs): weak estrogenic activity with some estrogen receptors (ER) was found. However, in these studies

antiestrogenic effects were also observed at concentrations two orders of magnitude lower. Such results show that caution should be exerted when interpreting the effects of endocrine disrupting chemicals.

Eisenhardt *et al* (2001) reported statistical associations between MX and MK levels in blood and gynaecological problems in women (due to potential reproductive toxicity and endocrine effects). The report must be treated with caution as no control group was used (the whole sample group consisted of women reporting to a hospital with fertility problems) and because the apparent associations do not in themselves provide proof of cause and effect.

With *in vitro* tests, Bitsch *et al* (2002) found a statistically significant increase in proliferation rate of human MCF-7 breast cancer cells associated with exposure to musk xylene, musk ketone and AHTN. Reduction of musk xylene to its 4-amine metabolite was found to increase the proliferation rate, such that half the concentration produced an equal effect to the parent musk. The converse was found to be the case for the only known major metabolite of musk ketone. It must be noted that all estrogenic effects observed were of a low level relative to oestradiol. HHCB and one of the four macrocyclic musks tested (muscone) were found to exhibit an estrogenic effect with such low potency that it was considered negligible. The other macrocylic musks tested, namely ethylene brassylate, ethylene dodecandioate, and cyclopentadecanolide, were not estrogenically active. Abramsson-Zetterberg and Slanina (2002) also found no genotoxicity associated with these three musks.

Evidence of potential co-mutagenic effects exists. Mersch-Sundermann *et al* (2001) found that in micronucleus tests with a human-derived hepatoma cell line (Hep G2), pre-exposure to musk ketone caused a dose dependant increase in the frequency of benzo(a)pyrene induced mutations. This indicates that musk ketone may potentially act as a sensitizing agent, increasing the risk associated with exposure to other environmental toxins, in a similar manner to that observed in marine bivalves (Smital *et al* 2004).

9. Regulatory position

Musk xylene has been on the OSPAR List of Chemicals for Priority Action since 1998. The latest OSPAR background document on musks concluded that musk xylene should be replaced in substances of a more favourable environmental profile. OSPAR stated that polycyclic musks "should not be promoted as suitable substitutes for nitromusks because, although not actually considered to be PBT-substances according to the criteria of the EC technical guidance document, they have unfavourable characteristics".

Although there remains very little information available on macrocyclic musks these compounds appear to be more environmentally benign. OSPAR suggest that macrocyclic musks may be acceptable substitutes, though through assessment of their environmental profile is needed (OSPAR 2004)

The definitive health risks of any particular chemical substance are always difficult, if not impossible, to quantify, and though they may take many years to complete, assessments are often highly subjective or even inconclusive. The assumptions used and judgements made in reaching conclusions regarding risks to the environment or human health are rarely communicated beyond technical papers, despite the importance of these aspects to the interpretation of conclusions drawn and the degree of uncertainty that underlies them.

Moreover, risk assessment starts from the position that some level of exposure to a chemical, even one showing intrinsically hazardous properties, is ultimately 'acceptable' and can be managed.

Given the added complexities resulting from the fact that we are exposed not to individual chemicals, but chemical mixtures and that there are commonly many different sources of each chemical in our daily lives, it is clear that traditional narrow risk assessment techniques are unlikely to provide adequate protection. A more precautionary approach to the evaluation and control of chemicals is urgently required.

The recent opinions adopted by the EU Scientific Committee on Cosmetic Products and Non-Food Products, SCCNFP (later reorganized into the Scientific Committee on Consumer Products in 2004), concerning HHCB and AHTN (SCCNFP 2002 a, b), illustrate the influence of underlying assumptions when determining "acceptable risk". The Committee advises that HHCB can be used as a fragrance ingredient in cosmetics without restrictions and that AHTN may be similarly used up to a maximum of 12% of the fragrance compound (as opposed to 12% of the final product), and base their recommendation on estimated 'margins of safety'. The calculation of these margins of safety depends heavily on the choice of representative values for exposure, skin absorption and toxicity.

In this instance, to determine the margin of safety for HHCB, the Committee assumed a skin absorption of 0.1% of applied dose and a typical concentration of the chemical in a perfume product (eau de toilette) of 2.4%. A much higher estimate of absorbed dose (5.1%) was rejected on the basis that the study from which it was derived did not meet the Committee's 'notes of guidance' and that it applied the dose in pure ethanol, considered unrepresentative of commercial products. But given that true perfumes (including Eaux de Parfum) can contain as high as 75% ethanol by weight (Bearling 1999), this study may have greater relevance. The results from the TNO analyses moreover show that even in comparably weaker eaux de toilette and eaux de parfum formulations, HHCB levels can exceed the Committee's assumption of a 2.4% concentration (in 5 of 36 products analysed by TNO). Applying these relatively 'worst case' estimates for absorption and concentration, margins of safety could have been reduced by a factor of at least 100.

For AHTN, the Committee similarly assumed a typical product concentration value of 0.96% and again rejected the consideration of a higher measure of absorbed dose in its calculations to determine a margin of safety. The results of the TNO analysis show how these values can be exceeded in 2 of the 36 samples tested. Additionally, the Committee itself cautions that its opinions on these chemicals do not take account of additional consumer exposure from a diversity of other sources (SCCNFP 2002 a, b). Cosmetics are not the only sources of human exposure to musks; air fresheners, soaps and laundry detergents may all contain musks.

In addition, it is unclear from the Committee's deliberations precisely how no-observed-adverse-effect levels of 50 mg/kg and 5 mg/kg for HHCB and AHTN respectively were derived, or whether these values include consideration of potential endocrine-disrupting properties or synergistic effects with other toxic chemicals. In any event, it is certain that new evidence of the environmental and health effects of these chemicals emerging since 2002 could not have been considered, though it may well be of relevance for the margin of safety calculated.

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