ELSEVIER

Journal of Chromatography B, 693 (1997) 71–78

JOURNAL OF CHROMATOGRAPHY B

Gas chromatographic method using electron-capture detection for the determination of musk xylene in human blood samples. Biological monitoring of the general population

J. Angerer*, H.U. Käfferlein

Institute and Clinic of Occupational, Social and Environmental Medicine, Schillerstrasse 25–29, D-91054 Erlangen, Germany

Received 16 August 1996; revised 14 November 1996; accepted 28 November 1996

Abstract

Musk xylene (2,4,6-trinitro-1,3-dimethyl-5-*tert.*-butylbenzene, MX), a synthetic musk often used in different fragrances and soaps to substitute the natural musk, is a potential contaminant of humans. In this publication, a specific and sensitive detection method for the determination of musk xylene in human blood samples is described. The clean-up of the blood samples includes an extraction step followed by a solid-phase adsorption to separate MX from other plasma components. Separation and detection was carried out by capillary gas chromatography and an electron capture detector (GC–ECD). The results were verified using qualitative capillary gas chromatography and a mass selective detector with electron impact ionisation (GC–EI–MS). ϵ-Hexachlorocyclohexane (ϵ-HCH) is used as internal standard. The reliability of the GC–ECD method has been proved. The relative standard deviations of the within-series imprecision were 12.7% for samples with a concentration of 0.5 μg/l and 2.1% for samples with a concentration of 5.0 μg/l, whereas the relative standard deviations for the between-day imprecision were 14.9% (0.5 μg/l samples) and 3.4% (5.0 μg/l samples). The losses during sample treatment were between 10.1% and 17.8%. No interfering peaks were observed. The absolute detection limit was 0.1 μg/l plasma. A total of 72 human blood samples were analysed to determine the MX concentrations within the general population. In 66 of the 72 human blood samples, the MX concentrations ranged from 0.10 to 1.12 μg/l plasma for the described method. In six samples no MX was detected. The median concentration was 0.24±0.23 μg MX/l plasma. The 95 percentile was 0.79 μg/l. No correlation could be found between MX concentrations and smoking habit, broca index, age, sex as well as fish consumption habits. Nevertheless, the results demonstrate the exposure of the general population to MX.

Keywords: Musk xylene

1. Introduction

Musk xylene MX (2,4,6-trinitro-1,3-dimethyl-5-*tert.*-butylbenzene), a synthetic nitromusk compound, is the main representative of a large group of nitroaromatic substances, which all have a musk like odour. Therefore, it is widely used as a substitute for the natural musk (Fig. 1) in perfumery and the detergent industry [1].

The main source of environmental pollution is sewage introduction [2]. MX was identified in freshwater fish [3,4] and several inshore waters [2]. The mean concentration of MX in freshwater fish was about 0.2 ppm on wet weight basis and the levels in the examined inshore waters ranged from 1 μg/l to 39 μg/l. Apart from a predicted intake of MX by the

*Corresponding author.

PII S0378-4347(96)00517-8

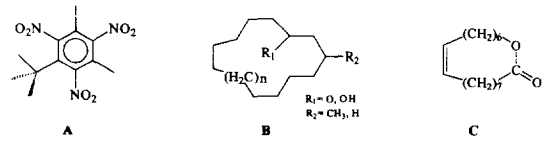

Fig. 1. Synthetic benzoic nitromusk compound musk xylene (A) is widely used as a substitute for two kinds of natural musks in perfumery: components of the scent gland secretion of the Asian musk deer (B) (macrocyclic alcohols and ketones) and musk ambrettolide (C), an extract of Indian and African musk marshmallows (cyclic lactone).

use of bodycare and perfumed household products [5,6], the consumption of fish may lead to a suspected ingestion of this substance in humans. Therefore, MX has been determined in mother's milk and fat tissue [7,8]. The MX concentrations in samples from mother's milk and human fat tissue ranged from 0.02 to 0.22 mg musk xylene per kg fat.

The acute oral and dermal toxicity of MX is low. The lethal doses are in the range of 10 g/kg body weight [9]. In animal experiments some hints for a nonmutagenic carcinogenic potential of MX were found [10].

Toxicological properties, high stability against biological and chemical degradation as well as high lipophilicity make MX an important environmental contaminant. There is much interest on the part of environmental medicine to determine MX in biological samples of the general population.

The main route of metabolism in rats is reduction of the nitro group in 2-position to the corresponding amine [11], but as MX is mainly excreted unchanged in faeces and urine [11], the determination of unmetabolised MX seems to be the best parameter for biological monitoring of environmentally exposed persons. The resulting values of the present study permit a first evaluation of internal exposure of individuals.

Concerning the legislation, there seems to be a lack of regulation needed for MX. This substance has not been reviewed by the international programme on chemical safety of the World Health Organisation.

We developed a simple and reliable analytical method for the determination of MX in human blood samples. This method, using capillary gas chromatography and an electron capture detector, was checked by qualitative analysis using a GC–EI–MS procedure. Using the GC–ECD method, the concentrations of MX in plasma of normal persons are reported.

2. Experimental

2.1. Materials

Silica gel (particle size 30–60 mesh) and *n*-heptane were obtained by J.T. Baker (Gross-Gerau, Germany); petroleum benzine, concentrated formic acid, anhydrous sodium sulphate p.a. and toluene were provided by Merck (Darmstadt, Germany); 2-ethoxyethanol was obtained by Fluka (Buchs, Switzerland); musk xylene (2,4,6-trinitro-1,3-dimethyl-5-*tert.*-butylbenzene) was obtained by Promochem (Wesel, Germany) and ϵ-hexachlorocyclohexane (ϵ-HCH) 10 mg/l in cyclohexane was provided by Dr. Ehrenstorfer (Augsburg, Germany); bovine plasma was obtained by Froschek (Mülheim, Germany). All chemicals and solvents were of analytical grade.

Glass columns (Bakerbond SPE, J.T. Baker) with a volume of 3 ml were filled with PTFE-frits (polytetrafluoroethylene), 700 mg 20% deactivated silica gel and a small layer of anhydrous sodium sulphate p.a. Before use the columns were rinsed with 5 ml of petroleum benzine. For solid-phase extraction (SPE) a Varian Vacelut SPS 24 station was used. The glass columns can be reused after SPE.

Silica gel was activated at 150°C for 24 h. Then it was deactivated by addition of 20% (v/w) water (high purity) of ASTM-type 1 and shaken for 2 h. After 24 h the silica gel is ready for use. It should not be stored longer than 3 days.

The bidistilled water (ASTM-type 1) and the conc. formic acid should be stored under petroleum benzine at room temperature. The MX containing solutions should be stored and transported only in glassware which had been washed with bidistilled water and acetone and decontaminated by heating.

Standard solutions were prepared for the calibration of the developed method. The pipetting scheme of the calibration standards in bovine plasma is shown in Table 1.

Starting solution 1: 10 mg MX is dissolved in

Table 1
Pipetting scheme of the calibration standards in the range from 0.1 to 10.0 μg/l bovine plasma

Calibration standard	Concentration (μg/l)[a]	Pipetting procedure	End volume (ml)
1	10.0	0.2 ml stock solution A ad 20 ml plasma	20
2	5.0	0.1 ml stock solution A ad 20 ml plasma	20
3	1.0	0.2 ml stock solution B ad 20 ml plasma	20
4	0.5	0.1 ml stock solution B ad 20 ml plasma	20
5	0.1	20 μl stock solution B ad 20 ml plasma	20

[a] Concentration of the calibration standards in μg MX/l bovine plasma. Concentration of stock solution A=1.0 mg/l and stock solution B=0.1 mg/l.

toluene in a 100 ml glass volumetric flask (100.0 mg/l).

Starting solution 2: 5 ml of starting solution 1 is diluted to the mark with toluene in a 50 ml glass volumetric flask (10.0 mg/l).

Stock solution A: 1 ml of starting solution 2 is diluted to the mark with 2-ethoxyethanol in a 10 ml glass volumetric flask (1.0 mg/l).

Stock solution B: 100 μl of starting solution 2 is diluted to the mark with 2-ethoxyethanol in a 10 ml glass volumetric flask (0.1 mg/l).

Calibration standards with concentrations ranging from 0.1 to 10.0 μg/l are prepared from these standard stock solutions by diluting with bovine plasma.

Solution of the internal standard ϵ-HCH: 0.4 ml of a solution of 10 mg ϵ-HCH per litre cyclohexane are diluted to the mark with *n*-heptane in a 50 ml glass volumetric flask (40 μg/l).

All solutions are stable at −18°C for more than 6 months.

2.2. Gas chromatography–electron capture detection

GC was carried out on a Varian 3400 gas chromatograph fitted with a temperature programmable injector, an electron capture (^{63}Ni) detector ECD and a Varian DS 650 Data Station. The carrier gas was nitrogen at 25 p.s.i. (1 p.s.i.=6890 Pa). The ECD was maintained at 320°C. The injector was maintained at 260°C. Split injection with a split rate of 20 ml/min was used. For separation of the analytes a 60 m×0.25 mm I.D. DB-5 column with a phase film of 0.25 μm was used (J&W Scientific, Folsom, USA). 3 μl of the samples were injected. The column temperature was held at 180°C for 15 min, raised to 240°C at 5°C/min and then to 320°C at 20°C/min. That temperature was held for 15 min. Retention times t_R: ϵ-HCH, 21.55 min; MX, 22.49 min.

2.3. Gas chromatography–mass spectrometry

GC–MS was performed on a gas chromatograph HP 5890 Series II fitted with a mass selective detector HP 5972 and a split/splitless injector system HP 7673 operating in the splitless mode (Hewlett Packard, Waldbronn, Germany). The inlet purge off time was set to 1 min. Electron energy was 70 eV, emission current 400 μA, electron multiplier voltage 400 V. The GC–MS was interfaced with a HP Vectra VL 2 Data Station (Hewlett Packard). Samples analysed by GC–MS were injected on a capillary column with a stationary phase DB-17 (J&W Scientific). The length was 60 m with an inner diameter of 0.25 mm and a film thickness of 0.25 μm. Helium was used as carrier gas at a constant flow of 0.8 ml/min. The initial column temperature of 180°C was held for 15 min and programmed for rise to 240°C at 10°C/min, held at that temperature for 10 min and then raised to 280°C at 10°C/min. That temperature was held for 15 min. For qualitative trace analysis of MX in human plasma samples, selected ion monitoring SIM was used. Therefore, it was possible to check the GC–ECD peaks and to identify MX undoubtedly. The following ions were monitored: m/z=297 and 282 (musk xylene, t_R= 23.10 min); m/z=181 and 183 (ϵ-HCH, t_R=25.85 min).

2.4. Specimen collection and sample preparation

The blood samples (10 ml) were taken from the arm vein using a disposable syringe containing potassium EDTA as an anticoagulant. The plasma is separated by centrifuging the sample at 800 *g* for 10 min. It is transferred into an injection flask and stored at −18°C until sample preparation is carried out.

For deproteinisation 2 ml concentrated formic acid is added to 2 ml plasma. After the mixture has been shaken for approximately 30 s, it is extracted with 1 ml *n*-heptane by mechanically shaking for 10 min. After centrifugation at 4000 *g* for about 10 min, 800 μl of the organic phase is taken up and chromatographed on a silica gel column. Then MX is eluted with 10 ml petroleum benzine. The samples were percolated through the reservoirs under a light vacuum. The remaining pressure was approximately 900 hPa. During elution of MX the silica gel columns were not permitted to get dry. After elution the columns were dried under a slight vacuum. 100 μl of the solution of the internal standard (40 μg ε-HCH/litre *n*-heptane) are added. Next, the eluate was evaporated to 50 μl under a stream of nitrogen. The samples were stored at −18°C for GC–ECD and GC–EI–MS analysis. The sample preparation is summarised in Fig. 2.

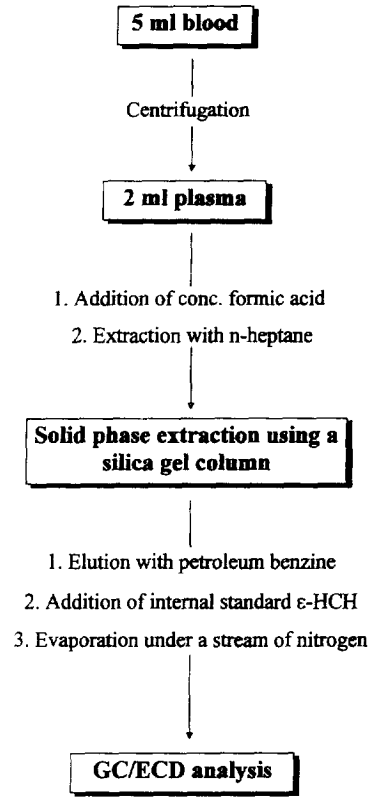

Fig. 2. Diagram of the clean-up procedure of human blood samples for determination of musk xylene.

2.5. Calibration process, calculation of the analytical result and quality control

The standard calibration solutions (0.1–10.0 μg/l) in bovine plasma are analysed as described. The quotients of the peak areas of MX and the internal standard are plotted as a function of the concentration. This calibration curve is used for the calculation of the MX content of each plasma sample. The concentration of MX is given in μg/l plasma.

As at present no quality control material is commercially available, it has been prepared in the laboratory. A known amount of MX is added to bovine plasma. This pool is divided into aliquots and stored at −18°C. A quality control sample as well as a blank bovine plasma sample is included in each analytical series.

In order to determine the within-series imprecision, bovine plasma was spiked with two different amounts of MX. Then the two pools contained 0.5 μg and 5.0 μg MX/l plasma. Plasma samples of both concentrations were analysed five times. In order to determine the between-day imprecision, samples of the same two plasma pools were analysed on five different days. The obtained results were the relative standard deviations for the whole method. In order to determine the losses which occur during sample preparation bovine plasma samples spiked with 5.0 μg MX/l plasma were prepared and processed as described in Section 2.4. Five bovine plasma samples were analysed. The results were compared with 10 ml of a standard solution of 5.0 μg/l MX in *n*-heptane which was analysed after addition of the internal standard and evaporation to 50 μl, but without further sample processing. The detection limit for MX was calculated from a signal to noise ratio of 3:1.

2.6. Collective

72 persons (30 male, 42 female) were investigated with respect to the MX levels in their plasma samples. 22 of the examined persons were smokers, 50 non-smokers. The age of the individuals ranged from 20 to 58. Furthermore, the persons were asked about their fish consumption habits, weight and height. No individuals were liable to any occupational contamination source due to MX. No data were available due to the use of body care and perfumed household products.

To evaluate the MX concentration results due to fish consumption habits, the examined persons were divided into three groups: Group 0, consisting of 17 persons, was consuming no fish. 31 individuals only ate fish approximately once a month (group 1) and 24 persons once a week (group 2). All plasma samples were investigated with the GC–ECD method and the resulting MX peak was verified by qualitative GC–EI–MS in the SIM mode to identify MX undoubtedly.

2.7. Statistical methods

For the statistical calculations and transformations SPSS for Windows was used. To determine statistical differences between two groups, non parametric Wilcoxon tests were carried out. To ascertain the correlations between MX concentrations and age, broca index [12], sex and fish consumption habits, multiple regression analysis was carried out. In the cases where MX levels were below the detection limit the value was set to half of the detection limit (0.05 μg/l) for the statistical calculations. Mean differences and correlation coefficients were calculated with 5 and 95 percentiles.

3. Results and discussion

For the quantitative analysis of MX in fish, human fat tissue and milk lipids clean-up procedures and determination methods are described. [2,4,8,13–18]. In all cases the concentrations of MX were usually determined by measuring the amounts of unmetabolised MX. However, these methods are not sensitive enough for the analysis of MX in human blood samples, which due to their lower lipid concentrations have much lower MX concentrations than, e.g., in fat tissue. Therefore it was necessary to develop a method which is suitable for routine determination of MX concentrations lower than 1 μg MX/l plasma.

3.1. Gas chromatography–electron capture detection

The reliability of the method has been proved. The relative standard deviations of the within-series imprecision of the described method range from 12.7% for samples with a concentration of 0.5 μg/l and 2.1% for samples with a concentration of 5.0 μg/l. The standard deviations of the between-day imprecision were 14.9% (0.5 μg/l samples) and 3.4% (5.0 μg/l samples). The losses during sample treatment were between 10.1% and 17.8% at a concentration of 5.0 μg/l. Under the analytical conditions described in Section 2.2 the detection limit is 0.1 μg MX/l plasma. No interfering peaks were observed, neither in plasma nor in blank samples. The developed calibration curve is linear between 0.1 μg and 10.0 μg MX/l plasma. More than 20 blood samples can be determined during one day with our sample processing and gas chromatographic analytical procedure. This makes the method particularly suitable for routine analysis with regard to biological monitoring of MX in environmental medicine. The possibility of determining MX by capillary gas chromatography and electron capture detection offers a further advantage, because the application of GC–NCI–MS which has been described in the literature [19] could be omitted. The efficiency of the method essentially depends on three factors:

- the liquid–liquid extraction of MX from the biological matrix,
- the subsequent purifying of the sample using a simple silica gel adsorption,
- the specific and sensitive ECD detection after the capillary gas chromatographic separation,

As it turned out a single liquid–liquid extraction step was sufficient. Therefore, this procedure does

not require considerable time and effort. A lower analytical background is achieved by evaporating the samples under a stream of nitrogen, immediately. It was shown that the samples were contaminated with interfering substances, if the evaporation was carried out under vacuum. Non-compliance with this fact leads to considerable differences between the reliability data of MX. It is not open to any doubt, that ϵ-HCH is not an ideal internal standard. As ϵ-HCH is not eluted by petroleum benzine, the internal standard must be added after silica gel adsorption. ϵ-HCH will be eluted by the use of toluene–hexane 40:60, but simultaneously an increase of the analytical background resulted in a higher detection limit. As solutions of 2,4,6-trinitrotoluene (TNT) are not stable for a longer period of time, the usage of TNT for internal standardisation is disadvantageous, too. Other substances tested (nitrobenzene and its derivatives, alkylated aromatic hydrocarbons, etc.) were also unsuitable for internal standardisation prior to the clean-up procedure as well. Nevertheless, although ϵ-HCH is added after SPE the precision of our method is within an acceptable range. This kind of internal standardisation is better than using no internal standard: variations in sample volumes during GC-injection as well as variations during evaporation of the samples with nitrogen will be eliminated. ϵ-HCH itself has further advantages: first of all the ECD shows excellent response to this substance. ϵ-HCH has retention properties similar to MX during GC–ECD analysis and does not occur in human plasma samples. Moreover, it is commercially available. The DB-5 capillary column used (as well as the DB-17 capillary column used in the qualitative GC–EI–MS analysis) gave good resolution of MX and ϵ-HCH and was suitable for the determination of MX in human blood samples. In our particular case a further increase in efficiency by the use of helium as carrier gas instead of nitrogen was not necessary. Therefore, we decided to use the less expensive nitrogen. To compensate for the disadvantages by injection of a higher sample volume (e.g., peak leading), split injection and higher column temperatures were used. This action resulted in better peak shape and higher signal to noise ratio than was achieved by using a splitless injection. A GC–ECD chromatogram of a human plasma sample containing MX is presented in Fig. 3. MX was eluted after 22.49 min and ϵ-HCH was eluted after 21.55 min.

As a whole this method for the determination of MX fulfils the requirements of environmental medicine for the surveillance of exposure to hazardous substances in biological materials.

3.2. Gas chromatography–mass spectrometry

MX was investigated by mass spectrometry. The spectra had a molecular radical ion m/z 297 $[M]^{+\cdot}$ and a fragment ion at m/z 282 ($[M\text{-}15]^{+}$). A concentration of 0.1 μg MX/l plasma was the detection limit using selected ion monitoring SIM for the fragment ion at m/z 282. The GC–ECD results were confirmed undoubtedly by the retention time of MX on the DB-17 column and the fragment ions described above. ϵ-HCH was investigated by mass spectrometry, too. All important fragment ions of the

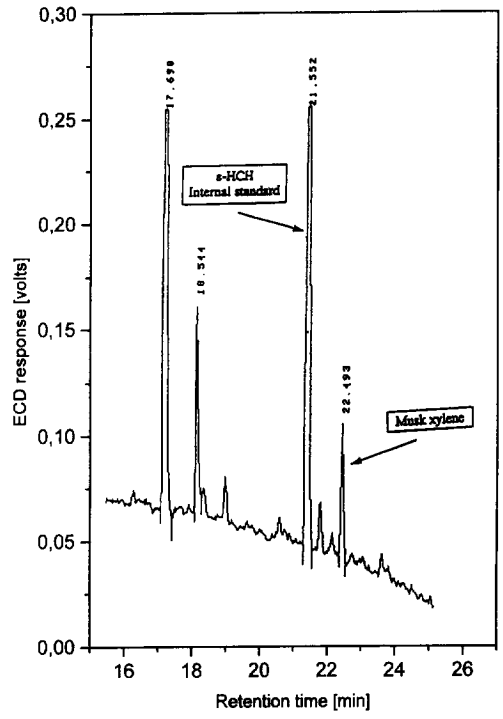

Fig. 3. GC–ECD chromatogram of a human blood sample containing 0.32 μg musk xylene/l plasma.

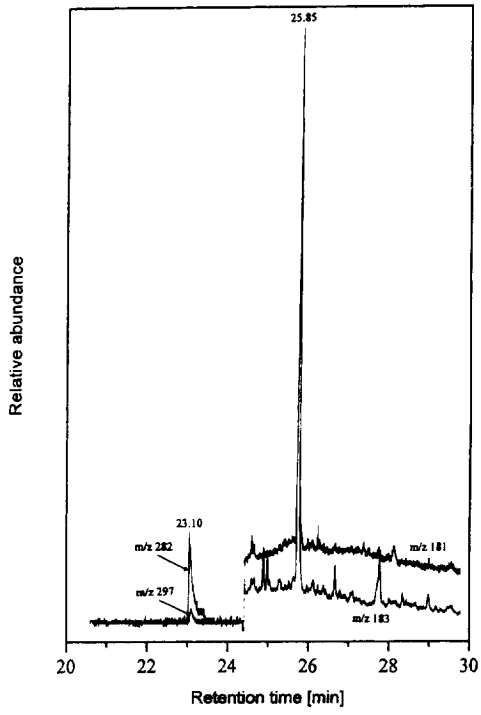

Fig. 4. GC–EI–MS chromatogram of a human plasma sample containing 32 μg musk xylene/l plasma. The four investigated ions were m/z=282 and m/z=297 for musk xylene (t_R=23.10 min) as well as m/z=181 and m/z=183 (t_R=25.85 min) for the internal standard ϵ-HCH.

internal standard ϵ-HCH result from the loss of different amounts of HCl molecules by the molecular radical ion. Therefore, two fragment ions of the

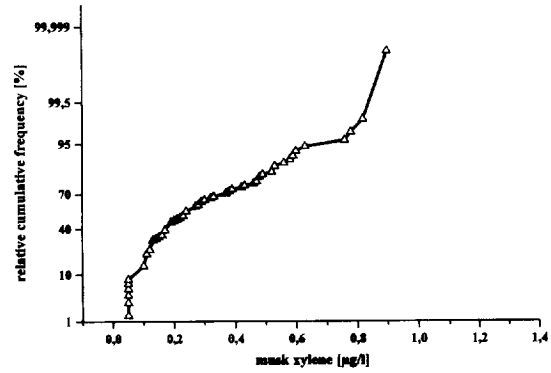

Fig. 5. Relative cumulative frequency distribution of all investigated musk xylene concentrations (n=72).

internal standard were investigated as well: m/z=181 and m/z=183. A GC–EI–MS chromatogram in the SIM setting of the four ions described above is presented in Fig. 4.

3.3. Evaluation of the human plasma samples results

The results of the analysis of the 72 human plasma samples are shown in Table 2. In 66 of the 72 plasma samples MX could be detected (91.7%). The MX concentrations ranged from 0.10 μg/l to 1.12 μg/l, respectively. The median level was 0.24 μg/l plasma. Fig. 5 shows the relative cumulative frequency distribution of all investigated MX concentrations. Our results are comparable to the published plasma MX concentrations by Helbling et al. [19].

Table 2
Evaluation of the 72 human blood samples

	Median level	95%	5%	Range	Standard deviation
Total (n=72)	0.24	0.79	0.05	0.05–1.12	0.23
Male (n=30)	0.28	1.00	0.09	0.05–1.12	0.25
Female (n=42)	0.20	0.76	0.05	0.05–0.82	0.21
Smokers (n=22)	0.21	1.08	0.06	0.05–1.12	0.26
Non-smokers (n=50)	0.26	0.77	0.05	0.05–0.90	0.21
Fish consumption group 0 (n=17)	0.27	0.63	0.05	0.05–0.78	0.22
Fish consumption group 1 (n=31)	0.20	0.85	0.05	0.05–0.90	0.22
Fish consumption group 2 (n=24)	0.30	1.03	0.05	0.05–1.12	0.25

All values are in μg MX/l plasma. 95%, 5%: percentiles. n=number of investigated individuals: 94 percentile.

This Swiss study analysed MX levels in the range of 0.066 to 0.270 ng/g plasma in nine human blood samples from three individuals.

First of all, the differences in MX concentrations between male and female individuals were checked. The MX amounts of male individuals (median level of 0.275 μg/l) seemed to be higher than the MX concentrations of female persons (median level of 0.200 μg/l), but this difference was not statistically significant.

Because of the high lipophilicity of MX (partition coefficient P_{ow}=5.2 [20]) and its determination in several biological samples, an accumulation within the food chain is expected. This assumption was supported by the fact that MX was detected in different freshwater fish. Therefore, we should expect higher plasma MX concentrations in persons eating fish than in those eating no fish. As we did not find any statistical relationship between MX concentrations and fish consumption habits, our assumption could not be confirmed.

Though, due to its lipophilic and persistent properties MX should be accumulated in human fat tissue. Nevertheless, no significant correlation was found between MX concentrations and the age of the examined persons.

Along with musk ambrette (another nitromusk compound) MX was found in indian chewing tobacco [21]. Therefore it seemed possible that MX levels could be higher in smokers than in non-smokers, but no significant differences between smokers (median level of 0.210 μg/l) and non-smokers (median level of 0.255 μg/l) were determined. Possibly, MX only seems to be an ingredient of chewing tobacco and not of cigarettes or (if cigarettes contain MX) the ingestion is small in such a way that it cannot be detected by our method.

In summary, we were able to establish a reliable method, which is sensitive enough to determine MX in human blood samples. According to our findings MX is environmentally taken up by the general population. However, we were not able to find the routes of uptake. This fact needs further investigation.

References

[1] D.P. Anonis, Perfumer Flavorist, 17 (1992) 23–29.
[2] J. Hahn, Dtsch. Lebensm. Rdsch., 89 (1993) 175–177.
[3] G. Rimkus and M. Wolf, Dtsch. Lebensm. Rdsch., 89 (1993) 171–177.
[4] T. Yamagishi, T. Miyazaki, S. Horii and S. Kaneko, Bull. Environ. Contam. Toxicol., 26 (1981) 656–662.
[5] C. Sommer, Dtsch. Lebensm.-Rdsch., 89 (1993) 108–111.
[6] T.J. Betts, M.T. Geraldine and R.A. Turner, J. Chromatogr., 244 (1982) 381–384.
[7] B. Liebl and S. Ehrenstorfer, Gesundheitswesen, 55 (1993) 527–532.
[8] M. Linkerhagner, H.J. Stan and G. Rimkus, HRC J. High Resolut. Chromatogr., 17 (1994) 821–826.
[9] D.L.J. Opdyke, Food Chem. Toxicol., 13 (1975) 875–881.
[10] A. Maekawa, Y. Matsushima, H. Onodera, S. Shibutani, H. Ogasawara, Y. Kodama, Y. Kurokawa and Y. Hayashi, Food Chem. Toxicol., 28 (1990) 581–586.
[11] K. Minegishi, S. Nambaru, M. Fukuoka, A. Tanaka and T. Nishimaki-Mogami, Arch. Toxicol., 65 (1991) 273–282.
[12] W. Pschyrembel, Klinisches Wörterbuch, 256th ed., 1990, p. 236.
[13] W. Specht and M. Tillkes, Fresenius Z. Anal. Chem., 301 (1980) 300–307.
[14] M.P. Yurawecz and B.J. Puma, J. Assoc. Off. Anal. Chem., 66 (1983) 241–247.
[15] G. Rimkus, B. Rimkus and M. Wolf, Chemosphere, 28 (1994) 421–432.
[16] M. Wolf, C. Eulig and G. Rimkus, Lebensmittelchemie, 48 (1994) 9–10.
[17] M. Green, Lebensmittelchemie, 48 (1994) 8.
[18] H.J. Geyer, G. Rimkus, M. Wolf, A. Attar, C. Steinberg and A. Kettrup, UWSF Z. Umweltchem. Ökotox., 6 (1994) 9–17.
[19] K.S. Helbling, P. Schmid and C. Schlatter, Chemosphere, 29 (1994) 477–484.
[20] Japan Chemical Industry – Ecology, Toxicology and Information Centre, Chemicals Inspection and Testing Institute Japan, Data of Existing Chemicals Based on the CSCL Japan, 1992.
[21] J. Nair, H. Ohshima, C. Malaveille, M. Friesen, I.K. O'Neill, A. Hautefeuille and H. Bartsch, Food Chem. Toxicol., 24 (1986) 27–31.