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A new version of an additive scheme for the prediction of gas chromatographic retention indices of the 211 structural isomers of 4-nonylphenol

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ABSTRACT

Control of environmental pollution by 4-nonylphenols (4-NP) and effective risk assessment concerning these xenoestrogens requires the identification of the individual isomers contained in the technical mixtures of 4-NP. A new approach is presented here which supports the identification of these compounds by a combination of experimentally determined gas chromatographic retention indices (*I*) of reference 4-NP isomers and calculated *I*-values. In addition to experimental indices, the *I*-prediction algorithm includes a new version of an additive scheme. The *I*-values of all structural 4-NP isomers are calculated on the basis of experimentally determined indices of a few available 4-NP isomers and the known retention indices of 75 iso-decanes. A mean deviation of ±11 index units between predicted and experimental *I*-values demonstrates the feasibility of the new approach. The predicted *I*-values provide information on the structure of 4-nonylphenol isomers in the technical mixture which has not been considered before. Furthermore, a novel line-coding system is proposed to describe the structure of isomeric 4-NPs and to initiate a current database for the endocrine-disrupting 4-nonylphenols.

1. Introduction

Environmental pollutants that induce certain estrogen receptor responses are known as xenoestrogens and are suspected of increasing the risk of developing cancer and reproductive abnormalities in wildlife species [1]. The 4-nonylphenols (4-NP), large scale products used as polymer stabilizers and in the synthesis of non-ionic detergents (nonylphenol polyethoxylates), are classified as xenoestrogens. Recognizing their estrogen potency, the European Water Framework Directive (2000/60/EC) included octylphenols and nonylphenols in the list of priority hazards that have to be monitored in the aquatic environment. Nonylphenol concentrations in ground and surface water range from a few ng/L to high μg/L values in sludge [2,3]. Although the industrial production and use of nonylphenols was banned from all applications in the EU within the last decade, their occurrence in the environment is still detectable and is decreasing only slowly. Due to its widespread occurrence in the environment, 4-NP is regarded as ubiquitous and has even been found in food [4]. In theory, the technical mixture of 4-nonylphenols can consist of 211 structural isomers [5–7] marked by different branched nonyl chains. Including diastereomers, a total number of 293 isomers results. This high number of isomers with quite similar properties causes problems in analysis. In particular, the evaluation of the risk potential of 4-NPs requires detailed knowledge of the isomer composition because toxicological investigations (yeast and E-screen assay) indicate structure-dependent estrogen receptor responses from individual nonylphenol isomers [8–10].

Isomer-specific analysis of 4-NPs is difficult, and not more than 44 isomers have been separated using GC–MS–MS in product ion scan or in selected ion monitoring mode [11,12]. Comprehensive two-dimensional GC in combination with time-of-flight mass spectrometry can improve the separation [13,14], but structure identification of individual isomers is still a challenge. Usually, synthesized individual nonylphenol isomers are added to technical 4-NP mixtures to identify the most abundant isomers. Interfering isomers are difficult to detect with these procedures [14–17]. Using gas chromatographic retention indices (*I*), a classic gas chromatographic tool, the elution order of 4-NP isomers can be predicted. Unfortunately, only few NP isomers are available as references for the experimental determination of *I*-values. Another strategy is needed to solve the problem of missing individual isomers.

Our new approach combines retention indices experimentally determined for reference 4-NP isomers with a prediction of *I*-values using an additive scheme based on the principles of structural analogy. The concept of a gas chromatographic retention index, first introduced by Kováts [18], allows the standardization of reten-

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tion data independently from varying instrument parameters, such as column temperature and carrier gas flow rate. The relative retention times of a set of *n*-alkanes are the reference basis to which the retention time of the analyte of interest has to be related.

The calculation of the retention index I_X of an analyte X involves the conversion of its retention time into an interpolated parameter presented in the "mobile" coordinate system defined by the retention times of reference *n*-alkanes:

$$I_X = I_n + (I_{n+k} - I_n)\left[\frac{f(t_{R,X}) - f(t_{R,n})}{f(t_{R,n+k}) - f(t_{R,n})}\right] \quad (1)$$

where $t_{R,n} < t_{R,X} < t_{R,n+k}$ are retention times of reference *n*-alkanes with *n* and $(n+k)$ number of carbon atoms in the molecules, eluted before and after the target analyte X; their corresponding *I*-values are set at $100n$ and $100(n+k)$. The function of the retention time $f(t_R) = t_R + q\log(t_R - t_0)$ provides the linear approximation of the dependence $I(t_R)$ at linear temperature programmed conditions; t_0 is the hold-up time of the chromatographic system, and the *q*-coefficient is calculated with retention times t_R for not less than three *n*-alkanes [19].

Structural features find expression in corresponding analyte-specific *I*-values. Differences between homologous compounds or between isomers become apparent as a systematic (incremental) shift in the GC retention. Calculating retention indices for structural variations can be simulated by simple additive schemes using a set of structural increments that enable the prediction of *I*-values of structurally related compounds. Therefore, pre-calculation of structure-dependant *I*-values can support the identification of unknown substances in chromatographic analysis.

However, the prediction of *I* for isomeric compounds requires another approach which must consider the conformational and intra-molecular interactions of functional groups and molecular sub-units [20]. In particular, interactions of vicinal functional groups or stereo-specific molecular requirements influence the gas chromatographic behavior of isomers. In order to consider most of the structural features in *I*-prediction, a new modification of an additive scheme was developed, which proved to be reliable for several classes of organic compounds. For instance, the *I*-values of all possible hydroxylated polychlorinated biphenyls (OH-PCBs, total number of congeners is 839) were predicted and used for the identification of OH-PCB isomers resulting from PCB degradation [21].

The objective of the investigations presented is to predict the *I*-values of all 211 structural isomers of 4-nonylphenols on a HP-5MS stationary phase. The retention order and the typical mass spectral features of the 4-NP isomers are proposed to support their structural identification. Additionally, a new numbering code for the 211 4-nonylphenol isomers is suggested to initiate a database for these endocrine-disrupting compounds.

2. Experimental

2.1. Material

A technical 4-nonylphenol mixture was purchased from Fluka (Buchs, Switzerland). For the investigations, a concentration of 1 μg/mL in *n*-hexane (Merck, Darmstadt, Germany) was prepared. The individual 4-nonylphenol isomers (marked in Table 5 by experimentally determined RI values) were synthesized as described previously [16,17]. The structures of these individual isomers were confirmed by ^{1}H and ^{13}C NMR. Their mass spectra were presented in publications [16,17] and served as references. The GC–MS analysis indicated the presence of 2–9% of the corresponding 2-NP-isomers which were formed during synthesis. The respective 2-nonylphenols are well separated from the major 4-NP isomers and do not affect any interpretation of mass spectra. Furthermore, diastereomers have to be considered. The mixture of *n*-C_8 to *n*-C_{20} alkanes used for the determination of the RI values was delivered by Merck. The *n*-alkanes diluted with *n*-hexane (Merck) were added to the individual NP isomers prior to GC–MS full scan analysis.

2.2. Determination of retention indices on 5% phenyl-dimethylsiloxane stationary phase

A "Polaris" GC–ion trap mass spectrometer (Thermo Electron, San José, U.S.A.) equipped with a 30 m HP-5MS capillary of 0.25 mm i.d. and 0.25 μm film thickness (Agilent Technologies, Palo Alto, U.S.A.) was used for the GC–MS analysis in full scan mode (50–550 amu). The GC separation started at the initial oven temperature of 50 °C (held for 3 min). The oven temperature was increased by 2 K/min to 220 °C (held for 10 min). For analysis, 1 μL of the individual isomer and of the technical 4-nonylphenol mixture, diluted 1:1000 with *n*-hexane, was injected in splitless mode into the GC at an injector temperature of 220 °C. Helium served as carrier gas at a constant flow of 1.2 mL/min. The ions were produced by electron impact ionization at 70 eV.

3. Results and discussion

3.1. New code system for the description of 4-nonylphenol structures

In cases of compound groups consisting of a large number of congeners, such as the 209 polychlorinated biphenyls (PCB), 75 polychlorinated dibenzodioxines and 135 dibenzofuranes (PCDD/F), a simple indication system for all the isomers is required that defines the individual compounds unambiguously. Apart from IUPAC nomenclature, numbering systems for the PCBs and PCDD/F congeners, proposed by Ballschmiter, have been established. The first empirical numbering system for nonylphenol isomers is related to their gas chromatographic retention order in a technical nonylphenols mixture [22]. The most intensive signals in the GC chromatograms were numbered consecutively, so that in many following papers NP1 to NP18 were used as isomers names. The interference from 4-NP isomers still not identified is completely ignored in this numbering system [9,14].

Following the Ballschmiter numbers for PCBs and PCDD/Fs, in 2006 Guenther et al. [7] proposed a numbering system for the 4-NP isomers. Starting from 4-*n*-nonylphenol as No. 1, all the NP isomers were consecutively numbered depending on their branching grade. This numbering system is difficult to apply because the list of corresponding NP structures represented by the numbers has to be known. A logical derivation of the structures from the numbers is not possible. Another system for NP abbreviations can be found in recent literature [16,17]. Particularly for the synthesized NP isomers, short names were created but not on the basis of IUPAC-related nomenclature.

To facilitate and clarify the representation, we propose a code system for 4-NP isomers that provides structure information directly from the code number (Fig. 1). The first number in the code indicates the length of the longest carbon chain in the nonyl substituent (from 4 to 9). The second number marks the positions of branching (from 1 to 8) and the number of carbon atoms in the sub-chains is given in parentheses (1, 2, 3, i3, 4, etc.). In Table 1, the new code numbers are listed together with numbering systems used previously. The advantage of the code system proposed is the simple and clear description of 4-NP isomer structures on the basis of the IUPAC nomenclature.