

**THE NEW STRATEGY AND FUTURE OF DIOXINS' ANALYSIS:
THE SUPPLEMENT OF NUCLEAR MAGNETIC RESONANCE (NMR)**

Fedorov Lev A.[a] and Dostovalova Valentina I.[b]

[a] V.I.Vernadski Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, 19 Kosygin Str., 117975 Russia, Moscow.

[b] A.N.Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 Vavilov Str., 117813 Russia, Moscow.

INTRODUCTION

The analysis of dioxins and especially dioxin-like compounds is restricted with their structural diversity¹.

The chromatographic determination of polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polychlorinated biphenyls (PCB) now requires the availability of 419 individual compounds, including 20 acutely toxic ones. The analysis of the corresponding 419 brominated compounds – polybrominated dibenzo-*p*-dioxins (PBDD), dibenzofurans (PBDF) and biphenyls (PBB) is more difficult but realizable.

The use of chromatography to study these compounds has been sharply limited with the discovery of mixed Cl,Br-containing dibenzo-*p*-dioxins (the total number 1700 compounds) and dibenzofurans (3320 compounds) suspected trace contaminants of specific chemicals and emissions from industrial incinerators and associated with combustion processes where organic materials and chlorine and bromine sources are present².

Practically nothing is known about the environmental occurrence of other mixed Cl,F- and especially more complex Br,Cl,F- compounds. The same concerns to S,O- analogues of polyhalogenated dibenzo-*p*-dioxins, S- analogues of polyhalogenated dibenzo-*p*-dioxins and dibenzofurans and other new polyhalogenated pollutants.

The formation of these pollutants are unavoidable for many Cl, Br- and F- technologies of the Russian chemical industry.

Thus it is now necessary to find a new effective approach to the analysis of thousands of dioxin-like compounds and their mixtures (with hundreds of extremely toxic ones), when the synthesis of all chromatographic reference compounds is impossible³.

METHOD

From the other hand ¹³C nuclear magnetic resonance (NMR) has already shown its advantage over other physical-chemistry methods in the structural determination of unknown organic compounds. Earlier it was shown⁴ that even in polysubstituted compounds ¹³C NMR chemical shifts can be related to molecular structures by using a

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limited number of parameters, and this allows one to reconstruct NMR spectra of all species of interest. Using an optimal experimental volume we obtained the reliable calculations of ^{13}C NMR spectra for all homostituted benzenes, polyoxybenzenes, related polychlorinated oxybenzenes and PCDD.

^{13}C NMR spectroscopy is one of the best techniques for the structure elucidation of unknown organic compounds because of its extremely high sensitivity to small structural changes. Contrary to one chromatography singlet requiring his own reference for the identification, ^{13}C NMR spectrum contains signals of all carbons in a molecule, and their positions do not depend in general on anything except molecular structure, so the ^{13}C NMR spectrum is a distinct portrait of the molecule.

It is evident that unambiguous prediction of ^{13}C NMR spectra for every structure can avoid of a great part of modelling syntheses. Usually this has been done by using particular increment schemes, which are suitable for studying some sets of homologues and isomers. Conventional increment schemes depend on the additivity of monoatomic influences, but such an approach is not acceptable for polysubstituted molecules because of complicated steric and mesomeric effects. The introduction of different sets of polyatomic increments is a hopeless task because of the great amount of parameters for real sets of compounds.

We suggest the new optimal strategy³⁾ of application of NMR ^{13}C to the dioxins field^{5,6)}. Two-particle increment schemes for ^{13}C NMR chemical shifts demanded a limited number of references for the modelling of all general structure situations for interesting classes of compounds and can be used to enhance the role of NMR³⁾ for analytical total problem solving. It is necessary to create new chromatographic procedures arranged with ^{13}C NMR for an identification of thousands compounds of dioxin-like classes. The approach must include the study of compounds from real mixtures and their structure determination with ^{13}C NMR, in order to obtain their chromatographic characteristics without the need for synthesis of reference materials.

RESULTS AND DISCUSSION

The procedure consist of a two-stage procedure of molecular structure correlation with the chromatographic signal for every compound:

- the synthesis of a few representative compounds to create an empirical increment scheme for calculating ^{13}C NMR chemical shifts predictive enough for reliable ^{13}C NMR spectra reconstruction for all compounds of a specific class;
- the extraction of the remaining compounds of the class from real mixtures by using preparative chromatography, followed by ^{13}C NMR measurements by using theoretical spectra for structure identification.

The difficulties caused because of different sensitivity levels of compounds to ^{13}C NMR and chromatography may be overcome by application of modern techniques.

It is important that such identification procedures be carried out only once for every compound. On the other hand, the powerful ^{13}C NMR technique for structure investigation requires a lot of time for every compound, especially if synthetic methods can not produce all of the required compounds as pure reference materials in a reasonable time-frame. For these purposes it is necessary to develop numerical methods of obtaining ^{13}C NMR spectra for all ecotoxicants and to create database of theoretical spectra.

That's why it is necessary to modify the analytical procedure for new classes of ecotoxicants – the chromatography must be accompanied with modern ^{13}C NMR as follows:

1. Creation of the method for theoretical ^{13}C NMR spectra calculations for organic aromatic compounds of the most important classes: polysubstituted benzenes with homogeneous substituents⁴⁾, different substituted benzenes, biphenyls (PCB⁷⁾ and PBB), naphthalenes⁸⁾ etc., using literature data.
2. ^{13}C NMR investigation of compounds with one type of substituents (excluding oxygen):
 - the application of polychlorinated oxybenzenes results for PCDD spectra calculations⁹⁾;
 - the measurement of ^{13}C NMR spectra of an optimal set of PCDD⁶⁾ and calculation of theoretical spectra for all 75 PCDD¹⁰⁾;
 - the measurement of ^{13}C NMR of an optimal set of PCDF and theoretical spectra determination for all 135 PCDF;
 - the same for all 75 PBDD and 135 PBDF.
3. ^{13}C NMR investigation of an optimal set of polyhalogenated (mixed Cl and Br) dibenzo-*p*-dioxins and dibenzofurans for the obtaining of the increment scheme for Cl,Br-dioxins.
4. ^{13}C NMR investigation of optimal set PCDD- and PCDF-like compounds containing S instead of O to obtain the corresponding increment scheme.
5. Application of the ^{13}C NMR spectra calculation method to other types of compounds – mixed polyhalogenated (Cl,F- and Cl,Br,F-) dibenzo-*p*-dioxins and dibenzofurans, polyhalogenated biphenylenes, azobenzenic and azoxybenzenic compounds etc.
6. Creation of special PC programs for the prediction of ^{13}C NMR theoretical spectra of dioxin-like compounds and for the comparison of each ^{13}C NMR spectrum with the set of theoretical ones.
7. Preparation of a database of theoretical ^{13}C NMR spectra for each compound in all classes of dioxin-like compounds.
8. The combination of chromatographic and ^{13}C NMR investigations: the ^{13}C NMR structure elucidation of any dioxin-like compounds, extracted from mixture with help of preparative chromatography.

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