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ScienceDirect

Procedia Food Science 5 (2015) 227 – 230

Procedia Food Science

International 58th Meat Industry Conference "Meat Safety and Quality: Where it goes?"

Methods for determination of dioxins and dioxin-like compounds – a brief review of recent advances

Radivoj Petronijevic[a,]*, Tatjana Radicevic[a], Vesna Jankovic[a]

[a]*Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade 11000, Serbia*

Abstract

Recent trends in development of methods for screening and diagnosis of environmental pollutants such as dioxin and dioxin-like compounds utilizing chemical and bioanalytical detection, their principles and advantages/limitations are described in the present literature review. This study briefly summarizes methods for determination of dioxin and dioxin-like compounds based on chemical methods, biological receptor ligands, enzyme-inducing compounds and artificial peptides.

Peer-review under responsibility of scientific committee of The 58th International Meat Industry Conference (MeatCon2015)

*Keywords:*dioxin; dioxin-like compounds; HRGC-HRMS; HPLC; bioassay; biomarker; dioxin toxic equivalents quotient (TEQ); polychlorinated biphenyls (PCBs); polychlorodibenzo-p-dioxins (PCDDs); polychlorodibenzofurans (PCDFs).

1. Introduction

The group of polychlorodibenzofurans (PCDFs) and polychlorodibenzo-p-dioxins (PCDDs) denominated as dioxins and dioxin-like compounds belongs to the chlorinated hydrocarbons family. The dioxin family, considering only molecules having chlorine as substituent in the rings, has over 200 compounds. They are highly toxic substances and very well-known environmental pollutants and carcinogens[1,2,3].

For a long time high-resolution gas chromatography/mass spectrometry (HRGC/HRMS) was the only option and

* Corresponding author. Tel.: +381-11-2650-655; fax: +381-11-2861-825.
E-mail address: rpetronijevic@inmesbgd.com

Peer-review under responsibility of scientific committee of The 58th International Meat Industry Conference (MeatCon2015)
doi:10.1016/j.profoo.2015.09.036

the standard method for detecting dioxin-like compounds. Enormous funds have been spent on determining the toxicity of PCDD/PCDFs in samples.[4] Thus, low-cost methods are needed to allow analyses of a large number of sediment samples in a short period of time. Therefore, in recent decades, advances in biotechnology have allowed the development of a number of in vitro bioassays and ligand binding assays for dioxin and dioxin-like compounds. By screening a large number of samples, the process not only saves money, but also improves the accuracy, reliability, and scientific basis for the quantitative assessment of environmental health risks. For several bioanalytical dioxin tests, official methods by governmental authorities have now been approved such as EPA Method 4425 (Reporter gene assay) or EPA Method 4025 (Immunoassay)[5]. The use of in vitro bioassays provides a useful tool as a prescreening method for dioxin toxic equivalents quotients (TEQs) in environmental samples.[6]

Recent bioanalytical detection methods (BDMs) for measuring dioxin-like activity are based on the ability of key biological molecules (e.g., antibodies, receptors, enzymes) to recognize a unique structural property of the dioxin-like compound, or on the ability of cells or organisms to have a specific response to dioxin-like compounds. In the last decade, artificial peptides were designed to mimic biological key molecules[3,7]. Therefore, the authors do not intend to give a complete overview of the available literature, but rather, to present the current state-of-the-art in bioanalysis for dioxin-like compounds.

2. Dioxin binding receptor and dioxin-like activity

Most bioassays for the determination of dioxins are based on the interaction of dioxin-related compounds through the aryl hydrocarbon receptor (AhR) signal transduction pathway. Dioxin-like compound binds to the AhR, the complex is translocated to the nucleus of the cell, where it induces the transcription of a number of genes, and subsequently, the production of proteins. Protein production includes cytochrome P-450 (CYP) 1A, an enzyme involved in oxidation, reduction, and hydroxylation reactions. The expression of a number of other enzymes is affected by exposure to dioxin-like compounds: an aldehyde dehydrogenase, an NADPH-quinone-oxidoreductase, etc. The AhR complex also affects the expression of other genes that influence basic cellular processes, such as growth, differentiation, and programmed cell death[8,9]. The initiation of changes in the expression of these genes begins with the ligand binding to the AhR.

3. Analytical methods for dioxin determination

The chemical methods used for analyzing dioxin-like compounds are primarily chromatographic methods (gas chromatography, GC or high performance liquid chromatography, HPLC) with various detection techniques (mass spectroscopy, MS, electron capture detection, ECD, photodiode array, PDA). The biological methods in operation included biomarkers, whole animal exposures (in vivo, laboratory exposure), cell- or organ-based bioassays (e.g., EROD, in vitro luciferase), and protein binding assays (e.g., ligand binding as well as immuno-assays).

It is unlikely that these biochemical screening methods will ever replace chemical instrumental analysis or in vivo toxicological studies. Instrumental analysis is necessary for the identification and exact quantification of the selected class of PHAHs, while the in vivo methods are necessary to study the bioavailability and prediction of whole-organism responses. However, the biochemical screening methods could complement chemical instrumental analysis and in vivo studies.

3.1. Methods of chemical analysis

These methods are based on the separation and quantification of dioxin-like compounds from matrices on the basis of differences in their molecular size, charge, mass, polarities, and redox potentials. The advantages are the structure conformation, the congener and pattern specificity, the calculation of the TEQ by the TEF-concept and international standardization. Disadvantages include potential loss in specificity, not all standards of interest are available, high cost, a long time for analysis, the limited information on the biological potency and potential interactions in complex mixtures of dioxin-like compounds[4].

The method of choice is HRGC with HRMS detection[10]. A few methods[11] are currently being used to rapidly extract and analyze for selected organochlorines in sediments, but these rapid methods either do not provide specific