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Polychlorinated dibenzo-p-dioxins and furans: methods of analysis, distribution in the Moscow region and application of biotesting methods to them

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Abstract. The review presents data on chemical-analytical methods of PCDD, PCDF determination and biotesting methods used for toxicological assessment of pollutants. Distribution of PCDDs, PCDFs on the territory of Moscow is analyzed. Analysis of publications of Russian and foreign authors showed that the currently existing methods of biotesting are in fact untested for dioxins. In order to quickly establish the toxicological effect of persistent organic pollutants in soil, it is suggested to use biotesting methods, with inclusion of test organisms representing the main trophic levels of ecosystems: producers, consumers, and decomposers.

Keywords: persistent organic pollutants, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, dioxins, chromatographic methods of analysis, biotesting, soil contamination

Authors' contributions: *E.A. Levashova* – literature analysis, preparation of the text of the article on chemical-analytical methods of analysis, biotesting methods. *S.E. Mazina* – conceptualization of research, critical analysis of the text. *G.V. Zykova* – analysis of literature on chemical-analytical methods of analysis of PCDD, PCDF.

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Полихлорированные дибензо-п-диоксины и фураны: методы анализа, распределение по территории Москвы и применение к ним методов биотестирования

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Аннотация. Приведены данные по химико-аналитическим методам определения ПХДД, ПХДФ и методам биотестирования, применяемым для токсикологической оценки загрязняющих веществ. Проанализировано распределение ПХДД, ПХДФ по территории Москвы. Анализ публикаций российских и зарубежных авторов показал, что существующие на настоящий момент методы биотестирования фактически не отработаны на диоксинах. Для быстрого установления токсикологического эффекта стойких органических загрязнителей в почве предлагается использование методов биотестирования, с включением тест-организмов, представляющих основные трофические уровни экосистем: продуцентов, консументов, редуцентов.

Ключевые слова: стойкие органические загрязнители, полихлорированные дибензо-п-диоксины, полихлорированные дибензофураны, диоксины, хроматографические методы анализа, биотестирование, загрязнение почв

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Introduction

One of the important environmental problems in the city environment is pollution by compounds with high toxicity, low solubility in water, spreading over long distances and being extremely resistant to decomposition. Such substances

include compounds from the group of persistent organic pollutants (POPs): polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF), commonly referred to as dioxins.

The difficulty in analysing these compounds is that the determination of trace amounts of contaminants occurs at the nano- and picogram (ppt, ppq) levels. In addition, PCDDs and PCDFs are large groups of substances in which only a small proportion are toxic and require measurement. To date, methodologies for the determination of POPs have been developed, registered in the Federal Information Fund for Ensuring Uniformity of Measurements and successfully applied. However, with regard to determination of PCDDs and PCDFs, today there are very few laboratories accredited for determination of these compounds due to high cost and complexity of analysis.

Areas of large cities, including industrial areas and their surroundings are the most exposed to contamination. It is particularly important to study soils as sites of accumulation, transformation and re-introduction of pollutants into biogeochemical cycles. To date, biotesting methods have been developed that make it possible to identify the most vulnerable links in the trophic chain and assess the mechanisms of action of pollutants on biota. A particular challenge is to investigate the hazards of different types of pollutants in biocosms, at different trophic levels.

The Stockholm Conference (2001) proposed a resolution on POPs[1], which was ratified in Russia only in 2011[2]. The need for Russia to recognize this convention has stimulated increased research into natural systems. In 2005, the Department of Nature Management and Environmental Protection of Moscow GPBU "Mosecomonitoring" carried out the first study of PCDD/PCDF in Moscow, which complemented standard analyses including pesticides, oil products, benz[a]pyrene and heavy metals. PCDDs/PCDFs were analyzed at 21 sites [1; 2]. Re-examination in 2012 of most of the previously investigated sites made it possible to identify possible sources of pollution.

Assessing the real picture of environmental pollution, in particular of soils, with the help of chemical-analytical methods alone is a difficult task. Therefore, the addition of biotesting on living organisms to such studies will help to obtain a more objective ecological assessment [3]. Literature sources show that existing POPs screening methods are virtually untested. In particular, biotesting methods, including very sensitive immunochemical methods, are insufficiently selective and specific [4].

In order to properly assess the biological effects of pollution, it is necessary to pay attention to what actually occurs in the field. Therefore, the most appropriate way of assessing environmental quality involves the use of species directly inhabiting the contaminated areas.

[1] Stockholm Convention on persistent organic pollutants. Stockholm. May 22, 2001.

[2] Federal Law No. 164-FZ of 27.06.2011 "On Ratification of the Stockholm Convention on Persistent Organic Pollutants" (In Russ.) [Федеральный закон от 27.06.2011 года № 164-ФЗ «О ратификации Стокгольмкой конвенции о стойких органических загрязнителях»]. Available from: http://ivo.garant.ru/#/document/12187282/paragraph/1:0 (accessed: 07.03.2022).

The purpose of the review is to identify existing problems in the analysis of POPs and to propose possible methods of PCDD and PCDF biotesting using the example of works on methods of analysis, determination of PCDD and PCDF content in Moscow soils and problems of biological control.

General information on contaminants

The term "dioxins" includes 210 compounds, of which 75 are PCDD congeners and 135 PCDF congeners. The most dangerous of them are considered to be 17 dioxin compounds having substituents in position 2,3,7,8 [5]. The most toxic representative is 2,3,7,8-TCDD which is characterized by high stability and shows mutagenic and carcinogenic activity [6]. The International Agency for Research on Cancer (IARC) categorizes 2,3,7,8-TCDD as group 1 (unconditional carcinogens for humans).

The main source of PCDDs and PCDFs in industrialized countries is considered to be high-temperature incineration of municipal solid waste, hazardous and medical waste. High production and release of PCDDs and PCDFs from waste incineration is mainly due to poor dust collectors in thermal waste incineration plants operating at elevated temperatures and poor combustion conditions. Often, incineration takes place in high-temperature incinerators at temperatures above 850°C. Thermal decontamination destroys the dioxins in the waste, but re-generates when the flue gas cools at 350°C. Some countries have passed laws that prohibit or restrict almost all methods of high-temperature waste incineration. There are no such restrictions in Russia.

Sources of dioxin emissions are power plants, industrial and domestic thermal energy plants, which are powered by fossil fuels or biofuels. Large quantities of PCDDs and PCDFs are generated during oil and natural gas combustion due to high calorific value of these fuels. It is known that the amount of PCDDs and PCDFs emitted in flue gases from large power plants is much lower than the corresponding values for coal- or oil-fired boilers and for domestic biofuel-fired boilers [7].

In internal combustion engines, dioxins are produced as a by-product of the combustion process. In Russia leaded petrol was used up to January 1 2003. High concentrations of PCDD and PCDF were observed due to the presence of chlorinated additives. With the use of unleaded petrol and a catalytic converter for exhaust gases in petrol engines, the concentration of emitted dioxins has been significantly reduced. In diesel engines, as a result of more efficient use of fuel, there is almost no emission of PCDDs and PCDFs.

It is known that as far back as in the 30s of XX century chemical productions started to develop which produced high concentrations of dioxins. Large territories are contaminated with dioxins during production of chlorinated cyclic organic compounds – chlorophenols, polychlorinated and polybrominated benzenes, biphenyls, as well as during production of pesticides – 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), pentachlorophenol (PCP). In Russia, most of the chemical industry enterprises leading to the formation and release of dioxins have been closed. The

production of 1,2-dichloroethane remains the only chemical industry generating PCDDs and PCDFs in Russia.

PCBs and PCDFs can enter soil by the introduction of PCB- and PCDF-contaminated products and wastes from industrial processes, by dioxin deposition in soil as a result of reactions in the environment and by deposition from atmospheric air.

Once in the soil, dioxins are firmly bound to the soil particles and do not practically migrate into the inner layers, being sorbed in the surface layer. The half-life in soils has been found to be about 9-15 years in the surface layer and 25–100 years at depth.

However, the presence of other pollutants in soil (e.g. oil products) may lead to dissolution of dioxins and thus to their more intensive migration into the inner layers. Some species of bacteria and fungi can degrade PCDDs and PCDFs, but this process is very slow. Their degradation is more rapid during photolysis.

Dioxins can enter the human body through inhalation of contaminated air, ingestion of contaminated food and skin contact with contaminated soil and other substances. Upon ingestion, most dioxins are absorbed through the gastrointestinal tract, are incorporated into biochemical processes, and accumulate primarily in adipose tissue and the liver. Dioxins are extremely stable, with a half-life of about ten years.

Mechanism of biological activity of dioxins is similar to the mechanism of action of many hormones-regulators of genome activity. The toxic effects of dioxins on the living organism are based on their interaction with a special cellular protein, Ah receptor (AhR), often referred to as dioxin receptor (DR), which controls the accumulation of non-specific monooxidases, cytochromes P-450 A1 and P-450 A2 in the body. Only 2,3,7,8-substituted PCDDs and PCDFs can exert their hormone-like toxic effects via the dioxin receptor system[3]. Disruption of regulatory mechanisms leads to weakening of the body's defenses against pollutants and suppression of the immune system.

Dioxins adversely affect the endocrine, nervous, cardiovascular systems, liver function and hematopoietic organs. In addition, the accumulation of dioxins increases the sensitivity of the human body to any anthropogenic action of environmental factors.

For toxicological evaluation of object contamination with dioxins a system of equivalent toxicity coefficients (TE) has been developed, i.e. determination of toxicity of each of dioxin congeners to toxicity of the most dangerous congeners – 2,3,7,8-TCDD. There are two basic systems of dioxin toxicity equivalents shown in Table 1: I-TEQ (DE) and WHO-TEQ (WHO-DE), which calculate the total toxic equivalent of any mixture of dioxins by multiplying the factor values by the concentration of each component of the mixture (Table 1).

[3] Toxicological profile for chlorinated dibenzo-p-dioxins. U.S. Department of health and human services. Agency for toxic substances and disease registry. Atlanta, GA, 1998. 721 p.

Table 1. **Toxic equivalents of PCDD and PCDF**

PCDD/PCDF	I-TEF	WHO-TEF
2,3,7,8-TCDD	1.0	1.0
1,2,3,7,8-PeCDD	0.5	1.0
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.001	0.0001
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.05
2,3,4,7,8-PeCDF	0.5	0.5
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.001	0.0001

In 2015 the hygienic regulation in soil for dioxins in Russia was revised and a decision was made regarding its relaxation (in 1986 the MPC value for dioxins in soil was 0.33 ng/kg – USSR MoH of 08.09.86 No. 697 DSP). At a meeting of the Rospotrebnadzor commission on rationing the MPC of dioxins in the soil of a residential area equal to 50 ng/kg was approved on 15 September 2015[4]. Norms adopted in foreign countries recommend the following criteria for normalization of dioxin content in soils [4] (Table 2).

Table 2. **Hygiene regulations for PCDD and PCDF in soil, ng/kg**

Type of soil	Russia	Germany	Italy	Nethelands	USA
Soil of agricultural land	5	5	10	10	27
Soil of populated ares	50	40	50	45	–
Industrial site soil	1000	–	250	–	1000

Methods of analysis

The main requirement for the determination of PCDDs and PCDFs is to determine the content of all 17 toxic congeners of PCDDs and PCDFs in the matrix.

Numerous international methods have been established for the determination of trace amounts of PCDDs and PCDFs in different matrices. They are based on effective purification of the substances to be determined from the background and include extraction, chromatographic separation and the instrumental analysis itself. In Russia, however, there are a limited number of laboratories capable of qualitative analysis of various environmental objects for PCDDs and PCDFs, as this type of analysis is extremely expensive, labour-intensive and time-consuming.

[4] SanPiN 1.2.3685 – 21 Hygienic standards and requirements for ensuring the safety and (or) harmlessness of environmental factors for humans, approved by the resolution Chief State Sanitary Doctor of the Russian Federation No. 2 dated 28.01.2021. (In Russ.) [СанПиН 1.2.3685 – 21 Гигиенические нормативы и требования к обеспечению безопасности и (или) безвредности для человека факторов среды обитания, утвержденные постановлением Главного государственного санитарного врача РФ от 28.01.2021 года № 2]. Available from: https://docs.cntd.ru/document/573500115 (accessed: 07.03.2022).

Literature analysis showed that for the determination of dioxins in soil, they are extracted by extraction in distillation-extraction apparatus (Soxhlet apparatus) with organic solvents, purified by TFE method and after concentrating the eluate, examined by high- and low-resolution gas chromatography-mass spectrometry (GC-MS) [8]. Due to the hydrophobic nature of PCDD and PCDF, almost any solvent not miscible with water can be used for extraction. The most commonly used solvents are acetone, methylene chloride, isopropyl alcohol and hexane, which due to its low polarity extracts the least number of polar impurities.

Extraction of solid samples in Soxhlet apparatuses can take from 5 to 24 hours, the organic solvents used are quite expensive and some of them are toxic. As an alternative to traditional Soxhlet extraction, extraction using supercritical water at 250°C and 50 atm is considered [9]. This method is considered to be simple, cheap, requires a minimum amount of time, and achieves an extraction rate of 80–85% of the target components from the soil [10; 11].

Recently, the accelerated solvent extraction (ASE) method has been quite common for the extraction of superecotoxicants from solid samples [12]. The relative simplicity of the equipment and the high promise have led to the rapid development of this method.

The literature [13] describes the technique and methodology of supercritical fluid extraction (SFE) for the extraction of PCDDs and PCDFs from solid samples and the advantages over conventional methods of contaminant extraction from the matrix.

Among other things, in order to increase the completeness of PCDD and PCDF extraction from solid samples, ultrasonic vibrations are applied to the sample [14].

The extracts obtained after extraction from soil samples need to be cleaned from the accompanying substances that may interfere with further instrumental analysis. For this purpose, various methods are used, recently consisting in multistage purification using chromatographic sorbents (aluminium oxide, siligacell, celites, etc.), which ensures extraction of all substances from PCDD and PCDF groups with content of the most toxic 2,3,7,8-TCDD at 1-10 ppt [12]. In the literature, purification of samples using activated carbon chromatography, which serves mainly to remove non-polar compounds, is also considered to be effective.

The choice of solvent for elution of interfering impurities depends on the properties of the sorbents. Mainly hexane, a mixture of methylene chloride with hexane, acetone, toluene are used for elution of PCDD, PCDF.

After purification of extracts on chromatographic columns with silica gel activated by alkali and acid, fractionation on column with aluminium oxide is carried out and then dioxins are concentrated on columns with activated carbon. The concentration technique is well developed. There are many techniques that describe the extraction and purification procedures [15–19].

Recently more automated high-speed systems combining extraction and purification of samples for determination of PCDDs, PCDFs are appearing. Automated systems such as ASE-150 and ASE-350 System (Dionex, Sunnyvale,

CA, USA) [13]; Total-Rapid-PrepTM (FMS, Waltham, MA, USA); Speed Extractor E-916 and E-914, Syncore Polyvap (Bünchi Labortechnik AG, Switzerland) [21]; Supelco Prep System (Sigma-Aldrich, St. Louis, MO, USA) [21]; Automated Sample Preparation Device SPD-600 (Ehime University and Miura Co, Ltd) [22] are mainly used for sample preparation in PCDD and PCDF analysis [23].

Reliable identification of isomeric dioxins and dibenzofurans is possible only in case of complete separation of the controlled components and using isotope-labeled (13C) standards and mass spectrometer as a detector [9].

The most universal method for the determination of PCDDs and PCDFs in soil samples is high and low resolution GC-MS [4].

Initially, low resolution GC-MS with quadrupole MS in the mode of selective detection of characteristic ions (SIM) was used for PCDD and PCDF analysis. The method has good sensitivity for dioxins, however, a multi-step careful sample cleaning procedure is necessary. Ionisation in this method can be carried out in two ways: electron impact and chemically (with the formation of positive or negative ions) [24].

Electron impact (EI) ionisation of a sample achieves a detection limit of 1–10 pg for TCDD and TCDP and 10-50 pg for OCDD and OCDP [25]. In chemical ionization of positive ions, their formation occurs under milder conditions than in ED, and the ions formed are more stable and the spectra are simpler [30]. Chemical ionisation of negative ions (CIC OI) provides a 1-2 order of magnitude increase in sensitivity compared to ED and chemical ionisation of positive ions [18].

Currently, the best methods for determination of PCDDs and PCDFs in complex matrices based on high resolution GC-MS [9; 12]. At 5000–10000 resolution a sensitivity of about 10–200 fg is achieved [25].

PCDD and PCDF isomers are separated on capillary columns made of fused quartz or glass [31] with the length up to 60 m. Different materials are used as fixed phase: strongly polar phase – Silar 10C, SP-2330, SP-2331, SP-2340, CPSil 88 and non-polar phase – DB-5, DB-17, DB-225, SE-54, etc [27; 28]. Different temperature regimes are also selected for best separation: from 120 to 270 °C at 20 °C/min, then from 240 to 270 °C at 2 °C/min and holding at this temperature until all congeners leave the column [4].

PCDDs and PCDFs are identified by the retention times and peak intensities of the characteristic ions of the identified congeners and the carbon isotope-labelled (13C) standards of PCDD and PCDF. Quantitative measurements are made using peak area ratios of the congener being identified to the corresponding peak area of the [11] isotopically-labelled imitator standard. The use of labelled standards, which provide an assessment of the separation efficiency and high accuracy of dioxin determination, is very important for dioxin concentrations of ppt and below.

Dynamics of PCBDD and PCDF distribution in Moscow

The study of PCDD and PCDF pollution in the soil cover started quite a long time ago, but the literature data on their content in the soil of large cities, and especially in Moscow, are very scarce [4]. There are very few data [4].

In 2005, the Department of Nature Management and Environmental Protection of Moscow State Unitary Enterprise "Mosecomonitoring" for the first time carried out a study of soils in the zone of influence of industrial enterprises and other sources for PCBDD and PCDF content [30]. In total, PCBDD and PCDF concentrations were determined at 21 sampling sites. Concentrations of PCDDs and PCDFs ranged from 0.27 to 57.3 ng DE/kg.

Further, a group of scientists [30] continued studies of PCDD and PCDF content in soils of different functional zones of Moscow. The average equivalent toxicity of the sum of PCDDs and PCDFs ranged from 0.27 to 48.66 ng DE/kg.

In addition to the listed works on determination of PCDDs and PCDFs in soils of Moscow, a study was conducted in 2012: the average value of PCDD and PCDF concentration in soils of Moscow was 5.5 ng DE/kg (observed levels are in the range from 0.35 ng DE/kg to 23.4 ng DE/kg) [31]. Pollution in residential areas is almost twice as low as in areas adjacent to industrial zones.

Exposure assessment using biotesting methods

At present, the most common method of assessing the environmental hazard of a particular pollutant is to determine it by chemical-analytical methods and compare the results with the established MPC values. The methods described above are rather labour-intensive, require expensive equipment, are material-intensive and are not always highly sensitive. It is known that it is not the levels of pollutants and exposures themselves that are important, but the biological effects they may cause, of which even the most precise chemical or physical analysis cannot provide information [32].

Many living organisms perceive fairly low concentrations of pollutants from anthropogenic sources and can be used to assess their toxic effects on biological systems.

Biotesting, along with the methods of analytical chemistry, has gained widespread use in international soil quality control in the last decade. In recent years, experience has been accumulated in this area in Russia as well [33–35]. Biotesting is usually understood as a procedure for establishing the toxicity of the environment using test objects [36]. There are several main approaches to biotesting: biochemical, genetic, morphological, physiological, biophysical and immunological [32].

Biotesting is based on the investigation of the efficiency of homeostatic mechanisms of living organisms, which are able to detect the presence of a stressor earlier than many commonly used methods [37].

Biotesting methods need to be informative, highly sensitive and operate in real time [38]. The greatest information in biotesting is obtained by assessing such parameters of organisms as survival, growth and fecundity. Test methods generally do not require complex sample preparation techniques such as separation and concentration [39]. The subject is extracted from the habitat, and the necessary analysis is carried out under laboratory conditions [32].

While animal experiments carried out to detect the carcinogenic properties of any chemical are a complex and costly scientific study, genetic tests, using a wide range of organisms, are considered to be more economical and allow results to be obtained within a few weeks [40].

Microorganisms are the most responsive to changes in the environment. Their development and activity are directly related to the composition of organic and inorganic substances in the environment, as microorganisms are capable of degrading compounds of natural and anthropogenic origin [32].

Algae underpin ecosystems and can therefore be used as bioindicators [36–38]. In [41], the possibility of using microalgae as a bioindicator species as an early detection of POPs in polluted sites was evaluated.

The alga *Chlorella vulgaris* is also a convenient object. It was in studies on this object in the 1960–1970s that N.P. Dubininin and V.A. Shevchenko established the main regularities of the mutation process dynamics in populations, which proved to be true also for other plant and animal species [40]. The studies were carried out on algae isolated from soil samples collected at the contrasting levels of radioactive contamination from the territory of the East Ural Radioactive Track. Already in the first experiments, it was shown that the frequency of visible mutations in *Chlorella vulgaris* increases with increasing radionuclide concentration in soil.

Most often for the detection of mutagenic chemical compounds are used tests using bacteria. This group of tests as a whole and most tested. Unlike eukaryotic organisms, in which DNA is organized into complex chromosomal structures, bacteria have only one circular DNA molecule, which is easily accessible to chemicals that penetrate through the cell wall [32]. A convenient test object is micromycetes, which develop directly in the soil.

Bacterial tests can be used to detect mutagenic metabolites in biological fluids of animals or humans exposed to chemicals. Thus, tests on bacteria, while not excluding or replacing studies on other objects, form their logic.

A test organism is considered to be a successful bio-indicator if the condition of obtaining information on the possible hazard of exposure before an environmentally significant disturbance occurs is fulfilled [40]. The use of plants to assess the presence of a wide range of pollutants, especially toxicants directly acting on target cell structures, is effective due to the fact that it is plants that constantly respond to a huge number of environmental parameters with high sensitivity. Due to their attached lifestyle, plants are constantly exposed to pollutants in the environment and characterize the ecological situation in their place of growth in the best possible way. Being at the base of the food chains, plants are exposed to toxic agents earlier than organisms of higher trophic levels. Plants have the ability to efficiently concentrate and transform substances in the environment, which increases the sensitivity and informational value of their use for environmental quality control. Higher plants can be effectively used in the field to assess air, water and soil quality and to evaluate the effects of chronic exposure.

Higher plant test systems can be combined with microbiological tests to detect promutagens [40]. Chromosomes and cell nucleus of plants, mammals and other eukaryotes are similar in their structure, functions, life cycle and react to the impact of mutagens in a similar way. Plant test systems can be used effectively under a wide range of environmental conditions.

Among higher plants, one of the most promising objects for the study of mutagenic factors is barley (*Hordeum vulgare* L.). Barley is an important agricultural crop that is widespread throughout the world. It can rightly be called one of the most genetically studied plants, intensively used in a variety of studies. The genus *Hordeum* belongs to the cereal family and differs from the other genera in the structure of the spike: its spikelets are monoecious. Most barley species are diploid (2n = 14), some species are tetraploid (2n = 28) or hexaploid (2n = 42). Cultured barley is diploid with seven pairs of chromosomes. The highest number of chromosomes and diploid structure facilitate genetic research, so barley often serves as a model plant. To date, several hundred genes with a variety of properties and characteristics have been characterized in barley.

Experiments on seed germination under the influence of pollutants such as oil are known in the literature [42–44]. The effects of dioxins on seed germination, and especially the mechanisms of their low concentrations, are scarce in the literature.

For cytological studies, it is very important to understand which organs and tissues of the plant are required. For example, mitosis can be observed in the meristems of young fast-growing plant roots, in the main roots of germinated seeds, and in the growth cones of the stem. Usually, to study mitosis and count the number of chromosomes in somatic tissues of plants, one prefers to work with young roots, because they have an active cell division zone (root growth cone) directly under the sheath and the division figures are conveniently oriented here [32].

The sustained interest in studying the mechanisms of toxic effects of pollutants on plants using the root apical meristem as a model system is due to the fact that it is the root tips that first directly contact with various chemicals in the soil [45]. The classic method for investigating the toxic effects of environmental pollutants on living objects is the onion root cell test (*Allium*-test), which allows a relatively rapid screening of chemical compounds with an indication of their potential biological risk [45]. An important advantage of this method of cytogenetic monitoring is the good correlation of its results with those obtained by other test systems [45].

A number of studies [46–49] have revealed that onion *Allium cepa* is a preferred test object for assessing the toxicity of anthropogenic xenobiotics.

The problem of low-dose ecotoxicometry of PCDDs and PCDFs has long been studied at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences. A.N. Severtsov Institute of Ecology and Evolution [50–52]. Dioxins in tissues of animals (rodents and fish) and in media (soil, sediments and snow) from biotopes near the Salarevo solid waste landfill were determined by GC-MS. Equivalent toxicity coefficient values were found to be

many times higher than the values at which the so-called dioxin pathology occurred in the population of dioxin-contaminated areas of the ecocide in Vietnam. Effects of contaminants on animals were reflected in the results of the study and evaluation of manifestations of toxic effects at the level of the whole organism and chromosome apparatus in relation to the parameters of dioxin and/or 2,3,7,8-TCDD content in their tissues and natural habitat. It has been established that the changes in the cytogenetic status in fish and rodents, morphometric parameters of the age development dynamics, appearance of individuals with abnormal structures among them corresponded to the pathological processes and states determining the pathogenesis of dioxin pathology.

To establish the permissible total doses of PCDDs and PCDFs coming from the environment and not affecting human health for a long time, studies [53] of dioxin contamination levels of food products produced in the Irkutsk region were conducted and the degree of PCDD and PCDF cumulation was studied. A high degree of cumulation of xenobiotics in lipoid samples was revealed. The maximum total concentration of dioxins was found in fat-containing products. Potential intake of dioxins with food was calculated on the basis of dioxin levels in each specific product, taking into account its share in the daily diet of an adult. The total dose of dioxins entering the body with food was determined by summing up the amount found in individual foods that make up the average diet of the population.

Estimations of daily intake of dioxins showed that the dose of 26.2 pg/kg/day with a very limited set of foodstuffs was 2.6 times higher than the permissible daily dose of 10 pg/kg, determined for humans weighing 60 kg (on the basis of WHO recommendations). At the same time, 98.6% of xenobiotics entered with food, only 1.4% with water [53]. The high levels of dioxins detected in fish – up to 46 ng/kg – indicated a high risk to populations whose diets are dominated by fish. On the basis of calculations of daily intake of dioxins in the human body, risk groups can be distinguished: the population whose daily diet is dominated by fish and other animal products. A conclusion was made and a recommendation to limit the use of 2,4-D as an herbicide in the Irkutsk region was given.

From 2003 to 2012 a longitudinal cohort study was conducted in Chapaevsk to assess the impact of dioxins and other POPs on physical and sexual development of boys [54]. For this purpose, a cohort of 516 families was formed and annual observation of a set of children's health indicators over a long period of time was organized using standardized methods of examination. The examination of children included biomonitoring of POP in diagnostic biosubstrates (whole blood, serum, blood clots – blood cells, urine, breast milk). The availability of such a databank makes it possible to carry out additional studies, to assess the impact of new risk factors on a particular health indicator long after the samples have been collected. The correlation between serum levels of PCDDs/PCDFs, PCBs and physical development indices in 8–9 year old boys has been established.

According to the results of studies [55] of the content in soil and needles of pine trees *Pinus sylvestris* L. growing near JSC Ufahimprom, it was concluded that the needles of Scots pine have a large accumulating capacity; with the accumulation

of toxic substances morphological changes were observed. The trees showed a decrease in the length and weight of the needles, the appearance of pitting and apical necrosis.

A team of authors [56] proposed an enzymatic method for PCDD and PCDF determination, which can be used for combined or preliminary express analysis of environmental samples. The method involved a combination of a physico-chemical sensor (sensor) and a biosensor, called a biosensor. However, the method has a disadvantage – the enzyme is often denatured by chemical reagents used in sample processing. Therefore, the choice of an enzyme in the design of the biosensor was a key task. Biotesting of prepared model samples was carried out. Before that, the results of enzyme biotesting showed that this control method is not informative for xenobiotics which do not have acute toxicity but are carcinogens and mutagens, such as dioxins. In this work, a modified method combining the techniques used in the manufacture of overhead membranes and described for single-use biosensors based on planar electrodes was developed.

Toxicity assessment based on biotesting of water samples [56; 57] containing dioxins underestimates their hazard when calculating the dioxin equivalent because the peculiarities of dioxin metabolism in living organisms are not taken into account. The toxicity coefficient system does not include the potential for further conversions of dioxins, which occur through the action of enzymes directly in cells, where less toxic compounds may become more toxic from carcinogenicity and mutagenicity standpoints. The fact that dioxins, in addition to their direct action, also have a synergistic effect, reinforcing the toxic effects of other substances, is also not taken into account. Model samples simulating background content of phenol in Kuibyshev reservoir have been chosen as objects of research. They were prepared from distilled water, copper nitrate and phenol. Biotesting was carried out on slipper infusoria *Paramecium caudatum* and daphnia *Ceriodaphnia affinis*. Qualitative reaction for the content of dioxin compounds in the model solution was carried out with nitrogen-based indicators. Cytochrome P4501A1 enzyme isoforms were used in studies of biochemical decomposition of dioxins. At the end of the experiment and processing of the results, it was concluded that in determining the hazard of xenobiotics to living organisms, we should move away from the definition of acute toxicity and focus on the processes triggered by enzyme systems when they enter the cell of living organisms.

A team of foreign authors [58] carried out a toxic evaluation of PCDDs and PCDFs from sewage sludge compost using luminescent bacteria and a genotoxic evaluation of sewage and sewage sludge using the Vitotox™ test. In addition to these studies, dioxin-like effects on the endocrine system were studied using yeast cells. It turned out that not all of the biotests used were approved in the study, as the compost component itself, which is rich in organic nutrients, reduced the sensitivity of the biotests.

The toxicity of dioxins has been investigated by evaluating the effectiveness of advanced wastewater treatment technologies to reduce contaminants [59]. Dioxin-like effects have been investigated on pollutant-exposed fish using in vivo

determination of the enzyme activity of ethoxyresorufin-O-diethylase (EROD). It has been shown that toxicity to fish can be reduced by additional treatment of wastewater. Thus, it has been shown that the side effects of pollution in fish can be predicted by biotests.

A method has been proposed to assess the toxicity of paper products, food products and soil based on the structural and physiological state of microalgae cell populations [60]. Cultures of the freshwater green chlorococcal microalga *Scenedesmus quadricauda* (Turp.) Breb. were used as test objects, as well as bottom soil using cultures of *Scenedesmus quadricauda* (Turp.) Breb. and the marine diatom microalga *Thalassiosira weissflogii* (Grunow) Fryxell et Hastle as test objects. Comparing the results obtained for the toxicity assessment of paper products using a culture of the microalgae *S. quadricauda* according to its structural and physiological condition with the data of hygienic examination of paper samples, it was concluded that the use of microalgae cultures for toxicological assessment of paper intended for hygienic purposes is more promising and economical (accessibility of the test object, ease of its maintenance and cultivation, cheapness of the method) [61–63].

Conclusion

The analysis of publications of Russian and foreign authors showed that the present methods of biotesting are in fact untested for dioxins. There is an idea to use representatives of three main links in the food chain of biogeocenoses as test-cultures for express analysis: producers, consumers and decomposers. In order to quickly establish toxicological effect of POPs in soil, we propose to use some biotesting methods, including test organisms that represent main trophic levels of ecosystems. Thus, experiments for establishment of MPC values of dioxins were already conducted on consumes. The authors have proposed biotesting methods using producers and decomposers [61–63].

It should be noted that the existing methods of environmental monitoring, both physico-chemical and biological, have their limitations. Both these methods are complementary. Accordingly, a combination of them is necessary for a reliable assessment of the state of the environment. As biotesting has recently emerged as a modern scientific and applied field, its application to assess the toxic effects of PCDDs and PCDFs is relevant.

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