"Environmental Engineering" 10th International Conference
Vilnius Gediminas Technical University
Lithuania, 27–28 April 2017
http://enviro.vgtu.lt

eISSN 2029-7092 / eISBN 978-609-476-044-0

Article ID: enviro.2017.021
DOI: https://doi.org/10.3846/enviro.2017.021

Approaches to Assessment and Hazard Identification of Dioxins

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Abstract. In the Russian practice in the framework of environmental regulation sanitary measurements to assess the toxicity of the objects of the environment, which are based on the determination of standardized components concentrations and comparing them with the limit value, are widely used. But this approach doesn't allow assessing the degree of biological hazards for organisms. The biotesting method has been considered for assessing the safety of dioxin-like compounds. Dioxins can be formed out of control in the environment. Ultraviolet radiation accelerates the formation of dioxins, as it enhances the ability of a chemical reaction of chlorine. This phenomenon is well known in Russia, where the chlorination is a standard procedure of water treatment and disinfection of drinking water, and control of the content of chlorophenols is an optional procedure. Simulation of the formation of dioxins in the process of chlorination of water, containing phenolic compounds, was carried out. Process of dioxins transformation in living systems to more toxic metabolites has been described. Enzymes that are involved in detoxification of dioxins have been identified. According to the results of bioassay danger of water samples, containing dioxins, is underestimated, since it doesn't take into account specific features of metabolism of dioxins in living organisms. Under the action of enzymes in the cells the less toxic compounds can be converted into the more toxic in terms of carcinogenicity and mutagenicity. The system of determination of the dioxin toxic equivalency factor doesn't account for it. Thus, during determination of danger of xenobiotics in living organisms we should move away from the determination of acute toxicity and focus on the processes that are started by enzyme systems when a toxicant gets into cells of living organisms.

Keywords: dioxins, bioassay, danger to the environment.

Conference topic: Environmental protection.

Introduction

Dioxins (Figure 1) are a collective term for six-membered aromatic halogenated hydrocarbons containing two oxygen atoms in their structure.

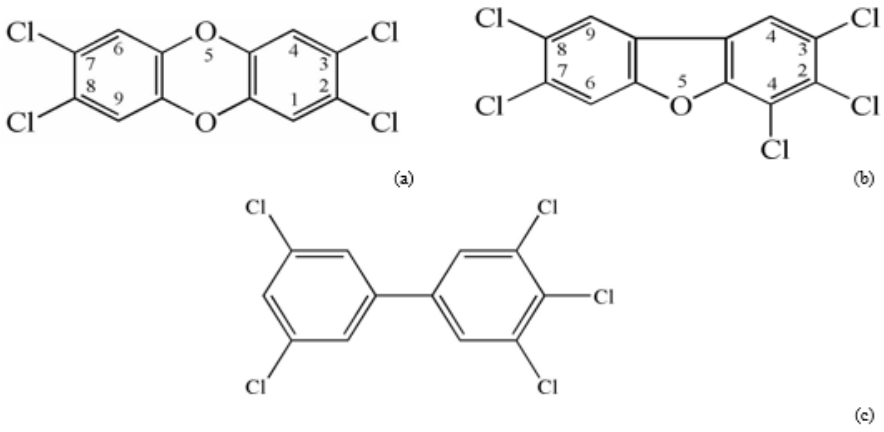

Fig. 1. The most dangerous representatives of the dioxin class: (a) polychlorinated dibenzo-p-dioxins, (b) polychlorinated dibenzofurans, (c) polychlorinated biphenyls

Dioxins and dioxin-like compounds are by-products of industrial processes (pyrolysis, pulp bleaching and so forth), chemical, petrochemical and metallurgical industries. They are also formed during the combustion of polymers (municipal and medical waste, wood). The environment contains dioxins in the form of complex mixtures, each of the components of which has a different toxicity. Therefore, in the samples of real objects of the environment it is difficult to determine their overall toxicity and assess the environmental hazard of the mixture (Ivshin, Polushin 2005). Dispersion in the atmosphere contributes to the transboundary transfer of dioxins, so their dissemination in the environment

is global: dioxins are found in the soil samples, bottom sediment, food products, water and air all over the world (Neubert 1997).

Ivshin and Polushin (2005) suggested that the formation of PCDD / PCDF is possible from aromatic compounds (precursors) in the presence of chlorine and catalysts (the transition metals). As precursors can serve aromatic compounds such as phenols, chlorophenols and chlorobenzenes. Copper chloride (II) can accelerate the conversion of aromatic precursors in chlorinated form and then into the dioxins and dibenzofurans (Gogol *et al.* 2013).
Humic and fulvic acids in the water are natural sources of phenolic compounds, which according to the above mechanism can be converted into compounds of dioxin series (Fig. 2).

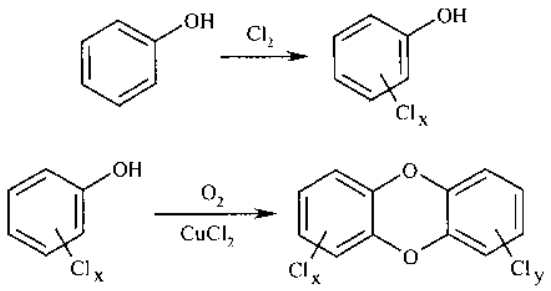

Fig. 2. Formation of dioxin-like xenobiotics from phenol as a precursor

All compounds of the dioxin series are characterized by a high melting point (>800 ° C), low water solubility (0.2 mg / l) and good solubility in nonpolar solvents, resistance to aggressive chemical environments, a high capacity for adhesion to all surfaces. Their accumulation or adsorption occurs in the bottom sediments, suspended solids, ash, soot and adipose tissues of the body. These properties are important for the prediction of formation of dioxins in the number of manufacturing processes and for selecting a method of analytical diagnostics (Alcock, Jones 1996). For selecting dioxin control methods are important not only physical and chemical properties of these compounds, but also understanding of the mechanism of their influence on living systems.

In the Russian practice in the framework of environmental regulation sanitary measurements to assess the toxicity of the objects of the environment, which are based on the determination of standardized components concentrations and comparing them with the limit value, are widely used. But this approach doesn't allow assessing the degree of biological hazards for organisms. Therefore bioassay is conducted on the test – organisms to characterize and evaluate the toxic effect.

By using the bioassay it is possible to determine the toxic effects of some substances that don't have the maximum allowable concentrations, and therefore their danger is not defined. Bioassay method allows us determine an integral toxicity is caused by the totality of all hazardous toxic substances and their metabolites which are present in the sample.

Based on these results, humans or animals reactions are predicted. With this approach to safety assessment it is difficult to get a forecast with a sufficient level of authenticity, because any biological models have varying degrees of approximation to the body, which is modeled. Consequently, the biological models have a different reaction to the products of metabolism of various substances inside the cell.

Necessity of using the bioassay for determining dioxin caused by the fact that the physico-chemical methods of analysis may be ineffective due to insufficient sensitivity. In addition, some congeners cannot be determined due to the lack of methods of determination and ultra-low concentrations. Living organisms are able to perceive a lower concentration of substances than any analytical sensor. That is they may be exposed to toxic influences that are not recorded by the technical equipment (Rozantsev, Cheremnyh 2003).

It is considered that only 17 congeners of PCDD and PCDF that have dioxin activity are dangerous. That is, they are very toxic to the environment. According to World Health Organization standards the toxicity of each compound of a dioxin series is determined by toxic equivalency factor (TEF) in relation to the most toxic form (2,3,7,8-TCDD). This is called dioxin equivalent (DE), which has a dimension ng / kg (Safe 1990):

$$DE = У(TCDDi* TEFi)+ У(TCDF i* TEFi)+ У(PCBi* TEFi) \quad (1)$$

Toxic equivalency factors are based on values of the acute toxicity derived from in vivo and in vitro researches. However, the method using TEF has its limitations, because the molecular organization of living is extremely difficult. Theoretically, any molecule of the organism can become a target for dioxin exposure. However, since the value of PTE is not the same for different congeners, the consequences of this influence are different.

The absence of acute toxicity of some compounds of a dioxin series doesn't say about the absence of danger of their impact on living organisms. The impact of congeners with low toxic equivalency factor or micro doses of congeners, which exhibit or don't exhibit acute toxicity, can be dangerous. The danger may be in the accumulation of these compounds, in connection with which may be formed cancers or genetic changes.

In addition, when dioxins fall into a living system, they are metabolized, in consequence of which are exposed to biochemical transformation under the influence of enzymes of a cytochrome P450 series (Gumerova *et al.* 2013). The cytochrome P450 enzyme system is found in all organisms, from bacteria, and is characterized by a high catalytic activity and the ability to oxidize almost all classes of complex organic xenobiotics (Guengerich, Ortiz de Montellano 2005). Mitochondrial cytochrome P450 are important to determine the intensity and time of action of foreign compounds. They are also important for detoxification of xenobiotics, as well as in their activation to toxic and / or carcinogenic metabolites (Nelson *et al.* 1996).

From a physiological point of view, this process is directed to protect living systems from the accumulation of these compounds in them. This process is an essential part of the adaptive response to foreign compounds that fall into the cell. And also it is important to enhance the detoxification function of the organism and excretion of xenobiotics (Gulyaeva, Grishanova 1994). However, in the case of the dioxins, metabolic by-products are phenolic compounds that can cause much greater toxic effects than native congeners. Moreover, acute toxic effects from products of metabolism of the dioxins will be different in all organisms. It depends on the activity of the enzyme phenol oxidase, which is able to decompose phenolic compounds and removes them from the organism. Thus the activation of the enzyme cytochrome P4501A1 is an indicator of the level of danger of dioxin-like compounds in relation to living systems.

Experimental part

Materials and reagents

As the objects of research were chosen the model samples, which simulated background concentrations of phenol in the Kuibyshev reservoir and were prepared from distilled water, copper nitrate and phenol.

To determine the concentration of phenol the standard samples of water and alcohol solutions of phenol (with an error of not exceeding 1%), crystalline phenol, sodium hydroxide, rectified ethyl alcohol technical, potassium chloride or distilled water, argon gas with an oxygen content of not more than 0.03 %, paper indicator universal, sodium bicarbonate (baking soda), filter paper were used.

The buffer solution was prepared based on the distilled water from disodium hydrogen phosphate, which contained ionic strength of 0.1 M Na_2SO_4 for constancy.

In order to simulate the formation of dioxins NaCl and NaOCl (freshly prepared) solutions, Fe_3^+ and Cu_2^+ chlorides were used.

The bioassay was performed on infusorians *Paramecium caudatum* and daphnias *Ceriodaphnia affinis*.

Qualitative reaction to the content of dioxin compounds in the model solutions was carried out with an indicator on the basis of nitrogenous compounds.

Solutions of a predetermined concentration of dioxins, which were used for the comparative analysis in determining the concentration of the model samples, were made from reference standard sample solutions of PCDD in toluene.

Isoforms of enzyme Cytochrome R4501A1 were used for the investigation of biodegradation of dioxin.

Simulation of the dioxins formation in the process of chlorination of water which contains phenolic compounds

Based on the fact that phenol is a precursor to the formation of dioxins in the first stage of the experiment three model samples (Table 1) were prepared

Table 1. The composition of model samples containing phenol

Number of the model sample	Composition	Laboratory Sample Conditioning	Appointment
1	Distilled water, $CuCl_2$, phenol	The phenol solution with a concentration of 0.001 mg/l was prepared	Imitation of background concentrations of phenol in the Volga River
2	Model sample number 1, chlorine gas	Chlorine gas is produced by electrolysis of NaCl and treated with it a model sample number 1	Imitation of disinfection of drinking water by chlorination during water treatment
3	Model sample №1, a solution containing NaOCl (household bleach), catalysts – Fe_3+ and Cu_2 +	The solution containing NaOCl (household bleach) was added to the model sample number 1 and treated with ultraviolet lamp	Imitation of conditions that are created by mixing wastewater with natural waters in summer

The yellow coloration while adding the indicator on the basis of nitrogenous compounds in the samples №2 and №3 has confirmed the formation of dioxin-like compounds from precursor – phenol. In the sample № 1 color reaction wasn't observed.

Determination of acute toxicity of model water samples containing dioxins and phenol by bioassay method

Acute toxic effect of the test water is determined by mortality of the test-objects during a certain period of exposure. The criterion of acute toxicity was the death of 50% or more infusorians and daphnias during 48 hours in the test water. In the control sample that is free from phenolic compounds, mortality of the test-objects shouldn't exceed 10%. The bioassay and determination of the toxicity criterion were carried out in accordance with (Federal Centre… 2006, Method T 14.1:2:4.12-06; T 16.1:2.3:3.9-06 2006; Method 1.39.2007.03221 2007; Temporary instruction № 247 2002; Meganorm 2007, Vasil'chenko *et al.* 2002).

Dilution of model samples №1, №2 and №3 was carried out according to Table 2. Additionally for each series of solutions the control samples with the cultivation water were prepared.

Table 2. Dilution factor of the aqueous extract depending on the hazard class

Class of hazard	Dilution factor of the aqueous extract at which the harmful effect on aquatic organisms is missing
I	>10000
II	From 10000 to 1001
III	From 1000 to 101
IV	<100
V	1

Results and discussion

Although there are many industries in which dioxins are a by-product, they may also be formed out of control in the environment under certain conditions. First of all, this applies to reservoirs that are polluted by industrial waste waters. The process is realized at a temperature from 20 °C. The impact of ultraviolet radiation accelerates the formation of dioxins, because it enhances chemical reactivity of the chlorine. This phenomenon is well known in Russia, where the chlorination is a standard procedure of water treatment and disinfection of drinking water, and control of the content of chlorophenols is an optional procedure. Indirectly, this phenomenon is reflected in the Russian standards of drinking water quality. In these standards permissible concentration of the phenol in nonchlorinated water is 0.1 mg/l, and in chlorinated water is 0,001 mg/l (Hakkinen *et al.* 2000).

If dioxins enter into the organism in doses which don't exceed the permissible, they don't possess an acute toxicity. Accumulating in the fatty tissues, they can lead to the long-term effects – to mutagenesis and carcinogenesis. This is evidenced by the results of bioassay of prepared model samples (Table 3). It was conducted to confirm the absence of acute toxicity of dioxin-like compounds and inability to determine them by screening – analysis on living systems.

Table 3. Results of bioassay of model samples №2 and №3

Type of the controlled object	Name of the test – object, the method	Experience type	Toxicity index	Class of hazard
Model sample № 2	Paramecium caudatum ERD FT 14. 1:2:3.13-06	Acute	$Kp_{(10)}$=30	4 (low-hazard)
	Ceriodaphnia affinis FR.1.39.2007.03221	Acute	$Kp_{(10)}$=70	
Model sample № 3	Paramecium caudatum ERD FT 14. 1:2:3.13-06	Acute	$Kp_{(10)}$=10	4 (low-hazard)
	Ceriodaphnia affinis FR.1.39.2007.03221	Acute	$Kp_{(10)}$=50	

Dioxin has to be activated with the formation of the electrophilic forms to manifestation of the toxic effect. As a result, the molecule is formed, which is capable of reacting irreversibly with nucleophiles of living tissues. Such biochemical activation is catalyzed by isoform of the enzyme cytochrome P4501A1.

All cytochromes P450 are heme-containing proteins. Usually the heme iron is in the oxidized state (Fe^{3+}). Monooxygenase reaction mechanism involves activation of oxygen to form highly reactive particles FeO^{+3}. The catalytic action of cytochrome P450 lies in the manifestation of a very strong oxidizing ability by FeO^{+3}. The labile iron is involved in the mechanism of toxic action, because it is included in the composition of proteins at an induction of enzyme family cytochrome P450. Thus, if the organism has an iron deficiency, the toxicity of xenobiotics is markedly reduced (Guengerich, Munro 2013).

Monooxygenase reaction that is catalyzed by cytochromes P450 is the primary, in which one oxygen atom is reacted with a substrate (ArH), while the other is reduced to HO^-. NADPH participates in the reaction as the reducing agent (2):

$$ArH\ (dioxin)+O_2 + NADPH + H^+ \rightarrow ArOH\ (phenol) + H_2O + NADP \quad (2)$$

The mechanism by which cytochrome receives an electron from the NADPH is dependent on intracellular localization of cytochrome P450 (Krest *et al.* 2013). According to Siberiak (2005), dioxin binds strongly with a special protein called the aryl hydrocarbon receptor (Ah-R), which a lot of cells of the organism have. Further this complex penetrates into the nucleus and activates specific genes. As a result, the content of isoforms of the enzyme cytochrome P4501A1 increases in cells. The excessive increase of the content of this protein accelerates the oxidation of molecules that important for the life of cells. This leads to disruption of many biological processes, and, moreover, increases the toxicity of other chemical compounds entering the organism. Essence of the work of cytochrome P450 is that it binds O_2 and recovers it in equivalent of active oxygen species. It is in this activated state, the enzyme oxidizes the bound substrate (Shkrob 1998). The main route of metabolism of dioxin-like compounds in mammals by cytochrome P4501A1 is their dehalogenation, cleavage of cycle and the formation of hydroxy – phenolic metabolites (Golikov *et al.* 1986; Claassen 2001), toxic effect of which was also confirmed by bioassay (Table 4).

Table 4. Results of bioassay of sample model №1

Type of the controlled object	Name of the test – object, the method	Experience type	Toxicity index	Class of hazard
Model sample № 1	Paramecium caudatum ERD FT 14. 1:2:3.13-06	Acute	Toxic, $Kp_{(10)}$=6000 $Kp_{(50)}$=5000	2 (highly hazardous)
	Ceriodaphnia affinis FR.1.39.2007.03221	Acute	Toxic, $Kp_{(10)}$=5000 $Kp_{(50)}$=2000	

The results of bioassay showed that acute toxicity is not a criterion of danger for xenobiotics such as dioxin. Therefore, this method of control for xenobiotics which don't have acute toxicity, but are carcinogens and mutagens, may be uninformative. The main danger from the compounds of this type is their long-term consequences. Their detrimental effect occurs only when they enter the body and enter into the metabolism under the action of enzymes.

Conclusions

According to the results of bioassay danger of water samples, containing dioxins, is underestimated, since it doesn't take into account specific features of metabolism of dioxins in living organisms. Under the action of enzymes in the cells the less toxic compounds can be converted into the more toxic in terms of carcinogenicity and mutagenicity. The system of determination of the dioxin toxic equivalency factor doesn't account for it. It is ignored that dioxins, along with direct action, increase the toxic effects of other substances. In addition, we cannot exclude the probability that in practice there are tasks that require registration of toxic contribution of not recorded factors.

Thus, during determination of danger of xenobiotics in living organisms we should move away from the determination of acute toxicity and focus on the processes that are started by enzyme systems when a toxicant gets into cells of living organisms.

References

Alcock, R. E.; Jones, K. C. 1996. Dioxins in the Environment: a review of trend data, *Environmental Sciense and Technology* 30(11): 3133–3143. http://pubs.acs.org/doi/abs/10.1021/es960306z

Claassen, C. D. 2001. Toxicology. *The basic Science of poisons*. 6th ed. New York, Chicago, Toronto, London. 1236 p.

Gogol, E. V.; Tunakova, J. A.; Gumerova, G. I.; Iskhakov, M. N.; Bogdanov, D. A. 2013. Express-analysis in environmental chemistry, *Scientific journal "Bulletin of Kazan Technological University"* 16(1): 163–167 [online], [cited 10 November 2017]. Available from Internet: https://www.google.ru/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=

0ahUKEwjizIyIot7JAhVGMnIKHXWrCA0QFggdMAA&url=http%3A%2F%2Fcyberleninka.ru%2Farticle%2Fn%2Fekspressanaliz-v-ekohimii.pdf&usg=AFQjCNGIn8cdjvztR-1IPA09gb3SCDYg_Q&bvm=bv.110151844,d.bGQ&cad=rjt

Golikov, S. N.; Sanotsky, I. V.; Tiunov, L. A. 1986. General mechanisms of toxic action, in *Medicine*. Leningrad. 286 p.

Guengerich, F. P.; Munro, A. W. 2013. Unusual cytochrome P450 enzymes and reactions, *The Journal of Biological Chemistry* 288(24): 17065–17073 [online], [cited 10 January 2017]. Available from Internet: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682512/

Guengerich, F. P.; Ortiz de Montellano, P. R. 2005. Human cytochrome P450 enzymes, in *Cytochrome P450: Structure, Mechanism, and Biochemistry*. Kluwer Academic/Plenum Press, New York, 377–530.

Gulyaeva, L. F.; Grishanova, A. Y. 1994. *Microsomal monooxygenase system of living organisms in the environment biomonitoring*. Analytical Review. State Public Scientific and Technical Library. Novosibirsk. 98 p.

Gumerova, G. I.; Galieva, A. T.; Gogol, E. V. 2013. Method of determination of dioxin-like toxicants, *Scientific journal Bulletin of Kazan State Technical University named after A.N. Tupolev* (1): 125–130.

Ivshin, V. P.; Polushin, R.V. 2005. *Dioxins and dioxin-like compounds: genezis, properties, methods of destruction* [online], [cited 10 January 2017]. Mari State University. Mari El. 320 p. Available from Internet: http://www.twirpx.com/file/696443/

Krest, C. M.; Onderko, E. L.; Yosca, T. H.; Calixto, J. C.; Karp, R. F.; Livada, J.; Rittle, J.; Green, M. T. 2013. Reactive intermediates in cytochrome P450 catalysis, The *Journal of Biological Chemistry* 288(24): 17074–17081 [online], [cited 10 January 2017]. Available from Internet: http://www.ncbi.nlm.nih.gov/pubmed/23632017

Federal Centre for the analysis and assessment of anthropogenic impact. 2006. *Method of determining the acute toxicity of drinking water, fresh water and sewage, water extracts from soils, sewage sludge and waste mortality of Daphnia. Federal environmental regulations T 14.1:2:4.12-06; T 16.1:2.3:3.9-06* [online], [cited 10 January 2017]. Moscow. 45 p. http://meganorm.ru/Data2/1/4293772/4293772638.pdf

Meganorm. 2007. *Method of determining the toxicity of water and aqueous extracts from soils, sewage sludge, waste mortality and fertility change of tseriodafnys. Federal Register 1.39.2007.03221* [online], [cited 10 January 2017]. Ltd. Akvaros. Moscow. 56 p. Available from Internet: http://meganorm.ru/Index2/1/4293842/4293842244.htm

Hakkinen, B. P. J, Kennedy, G., Stross, F. W. 2000. *Information Ressources in Toxicology*. 3th edition, Academic Press. 921 p. 10 January 2017Nelson, D. R.; Koymans, L.; Kamataki, T.; Stegeman, J. J.; Feyereisen, R.; Waxman, D. J.; Waterman, M. R.; Gotoh, O.; Coon, M. J.; Estabrook, R.W.; Gunsalus, I. C.; Nebert, D. W. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers, and nomenclature, *Pharmacogenetics* 6: 1–42 [online], [cited 10 January 2017]. Available from Internet: http://www.ncbi.nlm.nih.gov/pubmed/8845856

Neubert, D. 1997. Reflections on the assessment of the toxicity of dioxins for humans, using data from experimental and epidemiological studies, *Teratogenesis, Carcinogenesis, and Mutagenesis* 17 (4–5): 157–215 [online], [cited 10 January 2017]. Available from Internet: http://www.ncbi.nlm.nih.gov/pubmed/9508730

Rozantsev, E. G.; Cheremnyh, E. G. 2003. Bioassay or biological assessment of safety of present and future, *Ecology and Industry of Russia* (10): 44–46.

Safe, S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs), *CRC Critical Reviews in Toxicology* 21(1): 51–88 [online], [cited 10 January 2017]. Available from Internet: http://www.ncbi.nlm.nih.gov/pubmed/2124811

Shkrob, A. M. 1998. Drug and drove, *Chemistry and Life- XXI* (1–3) [online], [cited 10 January 2017]. Available from Internet: http://vivovoco.astronet.ru/HOME/PAPERS/TEXT/DRUGS/DRUGS_1.HTM

Siberiak, D. S. 2005. *Effect of super inducer of cytochrome P4501A1 2,3,7,8-tetrachlorodibenzo-n-dioxin on proliferative activity and apoptosis of lymphocytes*: thesis abstract on competition of a scientific degree of candidate of medical sciences [online], [cited 10 January 2017]. Chelyabinsk. 28 p. Available from Internet: http://medical-diss.com/docreader/256072/a#?page=28

Temporary instruction on the preparation of samples in the determination of the experimental method of waste hazard class for the environment № 247. 2002 [online], [cited 10 January 2017]. Ministry of Ecology and Natural Resources of the Republic of Tatarstan. Kazan. 10 p. Available from Internet: http://docs.cntd.ru/document/917010237

Vasil'chenko, Z. A.; Lyashenko, A. V.; Kovalev, V. I.; Agafonov, S. M. 2002. *Development of guidelines on the application of criteria for classifying hazardous waste classified as dangerous for the environment for the types of waste listed in Federal classification catalog of waste*. Ministry of Natural Resources of the Russian Federation: hands. Moscow. 41 p.