

The dioxin regulation criteria in the agro-industrial complex of Russia

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Abstract. The article studies the reactions of biochemical, hematological, pathomorphological, immunological and ultrastructural changes in the organisms of laboratory animals that were subjected to chronic dioxin poisoning at threshold doses were studied. There were held toxicological experiments on white rats of both sexes weighing 155–215 g in order to detect threshold doses. For two months, 2,3,7,8-tetrachlorodibenzo-para-dioxin (2,3,7,8- TCDD) in amounts corresponding to 1/100 and 1/200 LD 50. After each decade, some of the animals were euthanized and weighed to calculate the mass coefficient of internal organs. The obtained data comparison revealed no statistically significant changes in organ. Continued observation of animals in the experiment for up to two months, characteristic intoxication 2,3,7,8-TCDD clinical signs did not give. The correlation dependence of the minimum effective doses of 2,3,7,8-TCDD, namely 1/400 of LD50 with LD50, was determined. The obtained threshold dose of dioxin can be recommended as the basis for the normalization of dioxin in the Russian agro-industrial complex. There were determined biochemical, hematological and ultrastructural changes in the body of animals exposed to chronic poisoning of 2,3,7,8-TCDD in doses of 1/200, 1/300 of LD50. The article describes a statistically significant dependence of the respiratory activity of the liver mitochondria in laboratory animals (white rats and rabbits) on chronic poisoning with threshold doses of various degrees. Based on laboratory animals' experiments, threshold concentrations are determined and the maximum permissible levels of dioxin in the feed of some animals are tentatively calculated.

1 Introduction

The concentration of dioxin in tissues in the organs of farm animals in agricultural countries was lower than in industrial countries; So, in northern Vietnam – 145 ng/kg versus 1200 ng/kg in some areas of the United States. Dioxins were not found in the bottom sediments of lakes, which confirms the hypothesis of the anthropogenic origin of most dioxins [1]. Most researchers including Zheltov V.A. study the supply of dioxin from contaminated farmland to feed and in several studies reflects the level of dioxin replenishment in the components of the environment, its traffic along the trophic pathways water – feed – agricultural products – consumer. Food of animal origin is the predominant way of exposure to human dioxins due to their deposition in the lipid component of animal products. In lactating animals, dioxins are partially excreted with milk fat, and in laying hens, they are concentrated in the yolk content in the laid eggs.

Occupational exposure is a problem for some in agriculture, for example using chemicals, herbicides. Due to the ubiquity of dioxins, all people have a background effect and a certain level of dioxins in the body. However, due to the high toxic potential of this

class of compounds, there must be techniques to reduce the current background exposure. There is a special issue of toxicological picture of halogenated dibenzo-p-dioxins and dibenzofurans due to their presence in our environment and the high danger of several substances from these two classes of xenobiotics. Of great importance are 2,3,7,8-TCDD, it is estimated as the more dangerous of the compounds previously created by mankind [2].

The term dioxins itself is often used for a number of chemically related lipophilic compounds (polychlorinated dibenzo-para-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF), including some dioxin-like polychlorinated biphenyls (PCBs) with similar toxic properties. However, dioxin denomination is given to 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) Of the 419 types of dioxin-related compounds identified that have a dioxin-like chemical structure, only about 30 are considered significantly toxic, and TCDD is the most toxic.

Although dioxins and dioxin-like PCBs (hereinafter referred to as "dioxins") show similarities in their toxicological and chemical system, their sources can be different. Dioxins are common environmental pollutants. They are often called persistent organic pollutants due to their resistance to both physicochemical and biological

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degradation, decompose very slowly and remain in the environment for a long time because of their chemical stability. Dioxins, once introduced in biological organisms, including humans, accumulate in fatty tissues. For TCDD, the half-life in the body is estimated to be seven to eleven years, but other dioxins have a variable half-life. Dioxins have a highly toxic potential, because they affect several organs and systems and accumulate bio throughout the food chain.

Although the formation of dioxins is local in nature, the ecological distribution is global. Dioxins are found everywhere in the world in almost all ecosystems. The highest levels of these compounds are found in soils, sediments, and foodstuffs; dairy products, meat, fish, and mollusks are especially rich in them. Very low levels are found in plants, water and air.

Sources of dioxin contamination. Dioxins are produced as unwanted by-products as a result of certain types of business, plus certain manufacturing activities (for example, the production of chemicals and processes with high temperatures). Emergency situations at chemical synthesis plants cause the effects of emissions with a high concentration of dioxin and pollute local areas. The origin of dioxins can be household heaters by burning fuel, burning household waste of agricultural origin. Geological phenomena, such as volcanic eruptions and forest burning generates dioxins. In terms of the release of dioxins into the environment, incinerators (solid waste and hospital waste) are often the worst culprits, due to incomplete incineration and large amounts of incinerated waste.

The source of dioxins in the soil cover is atmospheric precipitation containing dioxins, transfer of toxic wastewater and sludge from sewage treatment plants to agricultural fields, dumping of sludge-polluted water onto pastures and excessive use of pesticides, and fertilizer contaminated with a toxicant.

The issue of control and regulation of this technogenic toxicant in agricultural production facilities has been raised with varying success in the sciences within recent decades.

2 Objects and research methods

The study used 2,3,7,8-TCDD with the active substance – 95 %. The experiments are based on rabbits and white rats.

The animals were fed food infected with 2,3,7,8-TCDD at concentrations of 1/100, 1/200, 1/300, 1/400 LD 50 for 60 days. Observations lasted 90 days. The clinical indicators were recorded in experimental subjects before the seed and every decade.

The respiratory activity of liver mitochondria was studied by the polarographic method on an LP –7E polarograph with a closed Clark electrode and a chamber to maintain a constant temperature. Ingredients of the incubation medium: sucrose 0.3 M, potassium phosphate disubstituted 10 mm, potassium chloride 10 mm (pH 7.5). Liver tissue was washed before homogenization to remove blood. This will allow you to get rid of NAD-oxidase of destroyed red blood cells. It was crushed in

the isolation medium in a ratio of 1: 7. The isolation medium consists of 0.25 M sucrose, 0.001 M EDTA, 0.004 M Tris-HCL, pH 7.5. Preliminarily, the tissues were passed through a 1 mm diameter press die. Mitochondria were isolated at 800 g centrifugation for 10 minutes and 9000 g for 20 minutes. Isolation of the mitochondrial substrate was carried out in the cold. Used reagents firms Reanal and Calbiochem. Clinical, hematological, biochemical and immunological parameters of animals were recorded according to generally accepted methods. The results were calculated by Student's t criterion.

The research purpose is to identify the dioxin dose threshold for calculating safe levels of TCDD in agricultural products; determine the possible deviations of the biochemistry, hematology and immunology indicators of experimental animals; to study pathological and morphological changes in the organs of animals in case of chronic poisoning of TCDD.

3 Discussion and analysis of the results

Man-made exposure provokes undesirable effects in the environment. Measures aimed at smoothing out these consequences require much time and financial issues. A possible solution to the problem, or one of the steps in this direction, may be measures based on observing the quality standards of environmental components, namely sanitary and hygienic requirements, through adjusting maximum permissible concentrations of technogenic ecotoxicants in agricultural products. This type of problem solving is called “zero damage” principle which is considered the main type in the world practice including Russia. We must strive to ensure that each toxic substance is given a dose at which changes in body functions will not be pathological or minimal. As our knowledge of the toxic manifestation of a pollutant on living organisms increases, and with the expansion of the boundaries of the measurement technique, the threshold for such an effect shifts, and the maximum permissible concentrations are specified. Assessment of the degree of exposure to a toxicant in certain environments is based on the following basic principles:

A) Each toxicant has a quantitative threshold of action; doses of the toxicant will be considered harmless at the threshold concentration level.

B) The values of maximum permissible concentrations are intended to protect each member of the society, and not the "average" person, from the pathogenic action of the normalized substance. Therefore, when normalizing, they proceed based on the groups or components of the system that are most sensitive to this substance.

C) The sanitary regulation of chemical pollution is based on in-situ observations and experimental data.

D) To determine the level of the threshold value of a toxicant, not only obvious pathological changes are considered, but non-specific functional changes and long-term consequences in the body are most important. Now in the ecosystem, both urbanized and agrarian, thousands of ecotoxicants are noted. It is not possible to

achieve their absence in food, feed, and it is necessary to reduce the degree of toxicity of living organisms. By regulating and observing these norms, the release of toxicants into the environment is limited [3, 4].

The standards for the safe presence of a toxicant in a component of the medium are fixed since multivariate analysis and toxicological studies. If we talk only about dioxins, then risks are assessed for them: immune, teratogenic, carcinogenic effects, etc.

The determination of permissible doses of toxicants into the body are formed on the following concepts. The non-threshold concept is that there are no threshold values for carcinogens. In the USA, this concept was taken as a basis and a mathematical model was calculated at the level of risk assessment. When evaluating dioxin, one additional case of the disease per one million people is considered, which for the daily intake is not more than 0.1 pg/kg of body weight. Another concept takes as a basis the generally accepted understanding of threshold doses. For super-ecotoxicants such as dioxin, the amount of daily intake of dioxin in the body is normalized in the range from 1 to 10 pg/kg of human body weight. When there is no threshold for harmful effects, such situations also occur in toxicology, then their presence in the environment will be scientifically justified.

The experiment was hold on laboratory animals: white rats and rabbits. Experimental animals are grown in vivarium. The diet of white rats and rabbits is a stationary diet in vivarium conditions. Groups of animals in the experiment were selected based on analogues. There were breed, age, gender and body weight characteristics considered. The number of studies and their type are given in Table 1.

Table 1. Conducted research, units

The research type	Number of studies (units)
Clinical	196
Hematologic	688
Immunological	344
Biochemical	344
Pathological	84
Histological	126
Polarographic	50
Analytical	10
Long-term effects	100

Toxicological experiments with the aim of identifying threshold doses were carried out on white rats of both sexes weighing 155–215 g. For two months, 2,3,7,8-TCDD there was added to the experimental diets in the amounts corresponding to 1/100 and 1/200 LD 50. After each decade, some of the animals were euthanized and weighed to calculate the mass coefficient of internal organs. The obtained data comparison revealed no statistically significant changes in organ mass. Continued observation of animals in the experiment for a period of up to two months, characteristic intoxication of 2,3,7,8-TCDD clinical signs did not give. The total cholesterol in the blood of animals that received dioxin at a dose of 1/100 LD50 did not statistically significantly

change; hemoglobin did not change significantly. On the 30th day of the experiment, hemoglobin of blood decreased by 10 %; the number of red blood cells in the blood for the first 10 days decreased by 20 % and came to a minimum during the 40–50 days from the day of inoculation. The erythrocyte sedimentation rate (ESR) of the experimental animals changed dramatically: thus, in the period from 10 to 20 days, the ESR doubled. The maximum value of the value characterizing ESR reached 50 days. Indicators characterizing the immune status of living exposed, chronic toxicity slightly decreased.

Analysis of the autopsy protocols on experimental animals describe the 20th day of seeding visible signs of damage to internal organs: the blood vessels of the liver are blood-filled; the liver contains uneven color, there are areas of dark red color; the spleen has no significant roundness of the edges. Small point hemorrhages were observed on the 30th day in the liver and cortical layer of the kidneys, in the same samples smoothing of the borders of the cortical and brain layers in the kidneys was noted. The 50th day of the experiment – sagging liver, in addition to the above lesions. On days 60–70, dark brown spots appeared on the spleen and its edges became dull. All the mentioned pathological changes in the organs persisted until the end of the observation [5, 6].

Assessment of the functional state of the mitochondria of the liver of animals with 2,3,7,8-TCDD intoxication, carried out by the polarographic method on rabbits fed with infected 2,3,7,8-TCDD at doses of 1/300, 1/400 LD 50, and on rats treated with food, infected 2,3,7,8-TCDD at doses of 1/200, 1/300, 1/400 LD 50 for rats for 10 days, are shown in Table 2.

Table 2. Change in the coefficient of increase in oxygen consumption by mitochondria of the liver of animals with 2,3,7,8-TCDD intoxication for 10 days, according to polarography with the addition of succinate (0.005 M) and ADP (200 μM), to mitochondria (3- 4 mg protein)

Experiment Conditions	Oxygen consumption acceleration	Activity (%)	Oppression (%)
White rats			
The control	1.4±0.1	100	
1/200 LD 50	0.3±0.1	21	79
The control	0.6±0.2	100	
1/300 LD 50	0.2±0.1	33	67
The control	1.6±0.1	100	
1/400 LD 50	1.3±0.1	81	19
Rabbits			
The control	5.9±0.2	100	
1/300 LD 50	2.2±0.2	27	63
The control	5.1±0.2	100	
1/400 LD 50	4.2±0.2	82	18

The respiratory activity indicator of the liver mitochondria of animals with 2,3,7,8-TCDD intoxication suggests that experimental animals compared with control animals provide a statistically significant decrease in the studied value. As shown in Table 2 in rats infected with dioxin dosed 1/200, 1/300, 1/400 LD 50, the oxygen consumption acceleration in the oxidative phosphorylation of succinate-containing substrate with the addition of adenosine diphosphate (ADP) decreased

by 78.5, 66.6 and 18.7 %. In rabbits dosed 1/300, 1/400 LD 50 – by 62.2 and 17.6 %, respectively.

We analyzed the change in the respiratory response to ADP of the liver mitochondria of 2,3,7,8-TCDD-intoxicated animals polarographically recorded, we examined the “state 4” before and after the administration of ADP in the respiratory reaction of isolated liver mitochondria of animals with various degrees of chronic intoxication with doses of 2,3,7,8-TCDD, judging by their nature, close to the threshold. The results obtained completed the chain of toxicological studies described above and confirmed our assumptions about the minimum effective dosed 2,3,7,8-TCDD during chronic intake of the animal with food. So, according to the research data for rats 0.15 µg/kg of weight at 10-fold intake and for rabbits, it is 0.075 µg/kg under the same conditions, which is 1/400 LD50 for these animal species.

According to the many researchers including Velom there is a discovery of a high correlation of 0.95 between the minimum effective doses and threshold doses. Thus, in experiments on pesticides, a relationship was obtained between the minimum effective doses for a period of 7 days (D7) and the threshold doses obtained by feeding them daily for 90 days (D90). The calculation showed that $D90 = 1/3D7$ if the doses are expressed as ED50 and $D90 = 1/6.2D7$ in the case of ED95. The minimum effective doses for a two-year duration of feeding are associated with the same at 7 and 90 days of feeding [2, 4]:

$$D2 \text{ year} = D90/1,8 = D7/5,4 \text{ dosed in ED50.}$$

$$\text{Dosed in ED95} - D2 \text{ year} = D90/5,7 \text{ or } D2 \text{ year} = D7/35,3.$$

The data obtained earlier not included in this article, do not allow us to speak of a pronounced teratogenic effect on animals at doses we operate on (hundredths of LD50), therefore we believe that under these conditions, dioxin does not have pronounced teratogenic effect [7].

Many dioxin-like substances are well absorbed in the digestive system of warm-blooded animals. However, highly halogenated isomers do not always show good absorption ability when they enter the body’s gastrointestinal tract. [4]. Perhaps the toxicity of dioxin, namely, its ability to pass into the cell, involves the transfer process from lipoproteins or from phospholipid vesicles, although this mechanism is not yet fully understood. The mechanism described above, apparently, is largely determined by the structure of the xenobiotic, the species characteristics of the animals, even the nature of the diet and the way it is kept, for example, the accumulation of adipose tissue.

When considering the intracellular distribution of 2,3,4,7,8-PCDF [2], it was found that labeled/C/PCDF accumulates in the liver of rats, and in the blood binds partially to lipoproteins, but more to albumin, and this binding enhances the inclusion of C/PCDF into hepatocytes, where a xenobiotic bound to cytochrome P-488 is located in the endoplasmic reticulum. The/C/PCDF binding form of the hemoprotein was found to be cytochrome P-450d, a product of the P-450IA2 gene.

We were able to obtain convincing data for determining the threshold doses by analyzing a polarographically recorded, altered reaction of the liver mitochondria respiration to the addition of ADP of 2,3,7,8-TCDD-intoxicated animals, also confirmed by electron microscopy data described in detail above. We investigated the “state 4” before and after the administration of ADP in the respiratory reaction of isolated mitochondria of the liver of animals with various degrees of chronic intoxication of 2,3,7,8-TCDD, close to threshold doses. The results show the calculated MACs given in Table 3.

Table 3. Estimated MAC 2,3,7,8-TCDD in the daily diet

Animal	MAC (mcg/ratio/day)
Rats	0.000005
Rabbits	0.000037
Chickens	0.000048
Pigs	0.24
Sheeps	0.037
Cattle	0.8

4 Conclusion

There is correlation dependence of the minimum effective dosed 2,3,7,8-TCDD determined, to be exact 1/400 of the LD50 with the LD50. The obtained threshold dose of dioxin can be recommended as the basis for the normalization of dioxin in the Russian agro-industrial complex. Biochemical, hematological and ultrastructural changes in the body of animals exposed to chronic poisoning of 2,3,7,8-TCDD at doses of 1/200, 1/300 of LD50 were determined. The article describes statistically significant dependence of the respiratory activity of the liver mitochondria of laboratory animals (white rats and rabbits) on chronic poisoning with threshold doses of various degrees. Based on experiments on laboratory animals, threshold concentrations were determined and the maximum permissible levels of dioxin in the feed of some animals were tentatively calculated. The values obtained by us are generally comparable with existing MACs. However, studies suggest that existing MACs should be reduced. To refine the MAC values by type of feed, information on the bioavailability of this xenobiotic from them is needed, which, apparently, is different for different animal species. The scientific literature presents wide range of uncertain data on this topic, so our research does not take it into account.

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