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TOXICOLOGICAL ASSESSMENT OF FOOD ADDITIVES BYBIOASSAY

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Abstract. The paper is devoted to the study of the toxicity levels of food additives by bioassay using *Daphnia magna*as model organisms. The directions and methods of research have been described. The toxicity of most food additives has been determined for the maximum permissible concentrations. The following widely used food additives have been tested: tartrazine (E102), sunset yellow (E110), azorubine (E122), Ponceau 4R (E124), indigo carmine (E132), brilliant blue (E133), sorbic acid (E200), potassium sorbate (E202), benzoic acid (E210), sodium benzoate (E211), formaldehyde (E240), sodium nitrite (E250), boric acid (E284), sodium isoscorbate (E316), citric acid (E330), phosphate Biophos 90 (E339), guar gum (E412), glycerol (E422), copper sulphate (E519), aspartame (E951), saccharin (E954). It has been proposed that the concentrations present in food products be used for bioassay of the additives Sodium Isoascorbate E316, Citricacid E330, Guargum E412, Glycerol E422, the maximum concentrations of which are not prescribed in the Sanitary Rules and Regulations. The paper determines the acute and chronic toxic effects that each food additive under study produces on *Daphniamagna*. For tartrazine E102, sunset yellow E110, Ponceau4R E124, and copper sulphate E519, no acute toxic impact was recorded, since the mortality of the model organisms did not exceed 25%. A synergistic effect of the use of popular combinations of food additives in various products has been revealed, namely that of: benzoic acid E210 and sorbic acid E200, sodium benzoate E211 and potassium sorbate E202, sodium nitrite E250 and sodium phosphate E339. The combination that caused the greatest lethality (more than 75%) during the bioassay was that of the additives E211 and E202. It has been established that all combinations of these food additives produce the greatest toxic effect, as compared with the toxic effect of individual additives. Further research in this direction has been proposed. The research results determine the need to reduce the maximum permissible levels of food additives that are currently in effect.

Keywords:food additive, toxicity, bioassay, testobject, mortality.

Introduction. Formulation of the problem

Nutrition as the only source of energy is the basic human physiological need. The quality of food products largely depends on standards, production technology, sources of raw materials, storage and transportation of finished products, and is one of the state's priorities of life quality and health of the population [1,2]. In Ukraine, the use of food additives is regulated by the Sanitary Rules and Regulations that define the requirements for food additives, enzymes, excipients in food production, and is approved by the State Register of Permitted Food Additives [3]. For each of them, the maximum permissible level (MPL) of a substance in a product is calculated based on the results of toxicological and other biological studies. Recommendations are made on the number of permissible daily intake of an additive with food, in accordance with official WHO documents and international practice. Compliance with the requirements of the Sanitary Rules is controlled by the local sanitary and epidemiological service following the relevant legislation of Ukraine. However, the expansion and dishonesty of manufacturers, the desire to succeed creates a certain pressure on the food market and results in the constantly increasing range and quantity of food additives used, often without taking into account their toxicity.

The importance of the research is due to a wide range of food additives, the use of which, being an environmental risk factor, contributes to the overall negative impact of chemical environmental factors. At the same time, the main criterion for assessing their quality has always been and still remains people's health.

Analysis of recent research and publications

The results of studying the regulation of using biotechnologies in industry, various aspects of biotechnological activities, biosafety, and bioethics have been published over the past two decades by A. Poddubny (2013, 2014), L. Bondar (2014), I. Girenko (2012, 2015), V. Zavgorodnya (2007), A. Krasovsky (2001), T. Korotky (2004), V. Kurzov (2007), G. Chebotarev (2009), K. Shahbazyan (2004), etc. The scientific works by such scientists as L. Lavrushenko (2013), V. Domaretsky (2009), P. Ponomareva (2014), V. Nykyforov (2013, 2015) are also devoted to this issue. Research by T. Gofrei, S.I. West (1996), C. Robinson (2001), A.D. Driffiths, D.S. Tawfic (2000), R. Breslow (2005), M. Zenkova (2004) concern the determination of the chemical properties of natural and artificial substances used, among other things, in the food industry [4-9]. However, the question of regulating the quality of food products in Ukraine remains open.

The signing of the Association Agreement between Ukraine and the EU and its member countries, which took place in 2014, obliges Ukraine to implement European standards in various areas of public life, including the sphere of water resources management, water protection and water pollution control. Similar standards are introduced in the EU Water Framework Directive (EU WFD), named Directive 2000/60/EC, of the European Parliament and the Ukrainian Parliament "On the establishment of a framework for the Commonwealth's activity in the field of water policy." This Directive dated 23 June 2000 establishes the main (framework) provisions for reaching the EU standards of good water in the water objects until 2015 [10]. According to these standards, bioassay of water is an integral component of determining its quality. Work by the following scientists, both local and foreign, are devoted to determining the toxic effects of natural and industrial wastewater on living organisms using biotesting methods: I. Martyniuk (2001), A. Vasylenko, V. Nykyforov, D. Salamatin, O. Sakun, L.Polishchuk, R.Dzierz, M.Kalimoldayev, Y.Amirgaliyev, S. Digtiar (2011, 2012, 2020, 2022), E.Strbova, P. Andrash (2011) [11-19]. However, the authors of this work are not aware of comprehensive studies of the effect of basic food additives on living test objects in this format.

The purpose of the research is to determine the impact of food additives in various concentrations and combinations on model organisms.

To accomplish this purpose, the following **objectives** have been achieved:

– the acute toxicity of widely used food additives has been determined using *Daphnia magna* Straus as the testobject;
– bioassay of the chronic toxic effect of aqueous solutions of food additives on model organisms has been carried out;
– the effect of combinations of individual food additives on hydrobionts has been investigated.

Research materials and methods

The following food additives widely used in foodstuffs have been tested: tartrazine (E102), sunset yellow (E110), azorubine (E122), Ponceau 4R (E124), indigo carmine (E132), brilliant blue (E133), sorbic acid (E200), potassium sorbate (E202), benzoic acid (E210), sodium benzoate (E211), formaldehyde (E240), sodium nitrite (E250), boric acid (E284), sodium isoascorbate (E316), citric acid (E330), phosphate Biophos 90 (E339), guar gum (E412), glycerol (E422), copper sulphate (E519), aspartame (E951), saccharin (E954) [6,7]. Samples of aqueous solutions of food additives were prepared in maximum permissible concentrations.

For bioassay of food additives having no MPC established by the sanitary rules and norms, average statistical concentrations of their content in food products were used (Table 1).

Table 1 – Concentrations of non-normative food additives under study

Name, code	Concentration, mg/dm^3
Sodium IsoascorbateE316	3000
Citric acid E330	5000
Guar gum E412	10000
Glycerol E422	3000

Currently, the methods most promising for quality control of food products are those involving analysis based on determining the mortality rate and changes in the fertility of biological objects (model organisms) [20-24]. The use of bioassay methods is regulated by DSTU 7525:2014 "Drinking water. Requirements and methods of quality control" and DSTU 4173:2003 (ISO 6341:2012) "Definition of acute lethal toxicity on *Daphnia magna* Straus and *Ceriodaphni aaffinis* Lilljeborg (Cladocera, Crustacea)".

The study has determined the acute and chronic toxic effects of aqueous solutions of food additives in concentrations equal to MPL on crustaceans *Daphnia magna* Straus. Acute toxicity was assessed in terms of mortality of crustaceans [25-27]. The effect of the low toxicity level (sublethal effect) was estimated as a chronic toxic effect and was detected over a longer period of observing the growth and reproduction of the test organisms.

The acute and chronic toxic effects that the aqueous solutions of food additives in concentrations equal to the MPL produce on the crustacean *Daphnia magna* Straus has been established.

Acute toxicity was assessed by crustacean mortality [24-33]. The effect of a low level of toxicity (sublethal effect) was estimated as a chronic toxic effect and was detected over a longer period of observing the growth and reproduction of the test organisms.

The acute toxic effect of aqueous solutions was determined by counting the number of model organisms *Daphnia magna* Straus that died in 24 hours. The criterion of acute toxicity was the mortality of 50% or more of *Daphnia* in the test solutions, the death rate in the control being 0%.

The chronic toxic effect of the studied food additives on the test object was determined by recording the dynamics of *Daphnia* fertility for 96 hours (before the appearance of the third litter of young fish in the control) in the samples studied. The reliability of the results was determined using Student's *t*-test.

The calculation was made according to the unified bioassay method used in Ukraine (DSTU 7525:2014) and its foreign analogues [23].

So, in accordance with the classification scale [34], the studied samples of aqueous solutions belong to the first class of toxicity, that is, they are non-toxic. The level of toxicity was determined based on the percentage of dead test objects (daphnia) in the test water compared with the control. As a control, tap water was used, which had been dechlorinated by two days' settling.

Comparing the obtained values of the actual level of toxicity with the standard of the maximum permissible level, we can conclude that the samples tested during the studies were mostly non-toxic or had weak or moderate toxicity. They met the standard of the maximum permissible level of toxicity, in accordance with the regulatory document "Methodology of determining the toxicity levels of surface and return waters to control their compliance with the normative regulations for water quality" [35].

In order to establish a synergistic effect, it has been investigated how the combinations of food additives affect *Daphnia magna*. The joint action of benzoic and sorbic acid, sodium benzoate and potassium sorbate, sodium nitrite and sodium phosphate has been studied. The additives were taken in the maximum permissible concentrations in the ratio 1:1.

Results of the research and their discussion

Table 2 summarises the results of determining the acute toxic effect (ATE) of food additives that accumulate in the body and violate its structural and functional organisation (organization – можеттак??) at the molecular level [33] and, consequently, at the cellular and organic tissue levels.

Acute toxic effects of the food additives on the test object were not identified, since the number of dead *Daphnia* did not exceed 25% (for tartrazine E102, sunset yellow E110, Ponceau 4R E124, copper sulphate E519). Aqueous solutions of azorubine E124, brilliant blue E133, formaldehyde E240, indigo carmine E132, saccharin E954, and sodium isoascorbate E316 in the maximum permissible concentrations caused the death of 20% of the test object. The remaining food additives led to the death of ten (aspartame E951, sodium nitrite E250, sorbic acid E200, benzoic acid E210, sodium benzoate E211, boric acid E284) and seven percent (potassium sorbate E202, sodium phosphate E339, citric acid E330, guar gum E412, glycerol E422) of *Daphnia* respectively [32,33] (Fig. 1).

Table 2– Results of the short-term bioassay of food additives

Index	Name	Function	MPL, mg/dm^3	ATE,%
E102	Tartrazine	Syntheticyellowdye	30	25
E110	Sunset yellow	Syntheticyellowdye	50	25
E122	Azorubine	Syntheticreddye	50	20
E124	Ponceau 4R	Syntheticreddye	50	25
E132	Indigo carmine	Syntheticbluedye	30	20
E133	Brilliant blue	Syntheticbluedye	25	20
E200	Sorbic acid	Preservative	500	10
E202	Potassiumsorbate	Preservative	500	7
E210	Benzoic acid	Preservative	150	10
E211	Benzoate	Preservative	150	10
E240	Formaldehyde	Preservative	0.001	20
E250	Sodium nitrite	Preservative, colour lock	3.3	10
E284	Boric acid	Preservative	0.5	10
E316	Sodium Isoascorbate	Antioxidant	–	20
E330	Citric acid	Acidity regulator, antioxidant	–	7
E339	Sodium phosphates	Acidity regulator, emulsifier	4000	7
E412	Guar gum	Thickener, stabiliser, emulsifier	–	10
E422	Glycerol	Thickener	–	7
E519	Cuprum sulphate	Preservative, colour lock	0.1	25
E951	Aspartame	Sweetener	600	10
E954	Saccharin	Sweetener	80	20

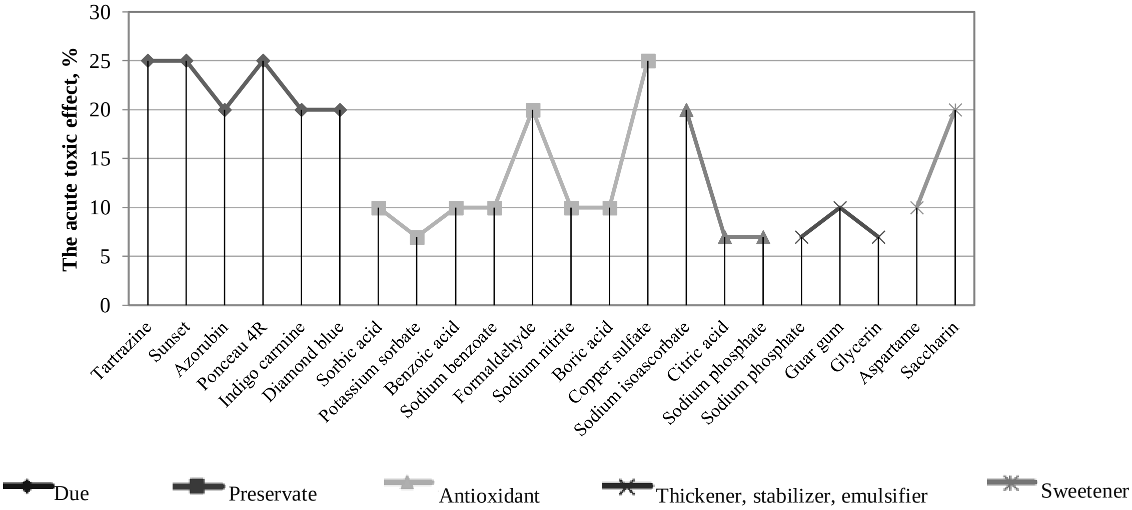

Fig.1. Mortality levels of model organisms in aqueous solutions of the main classes of food additives, where:

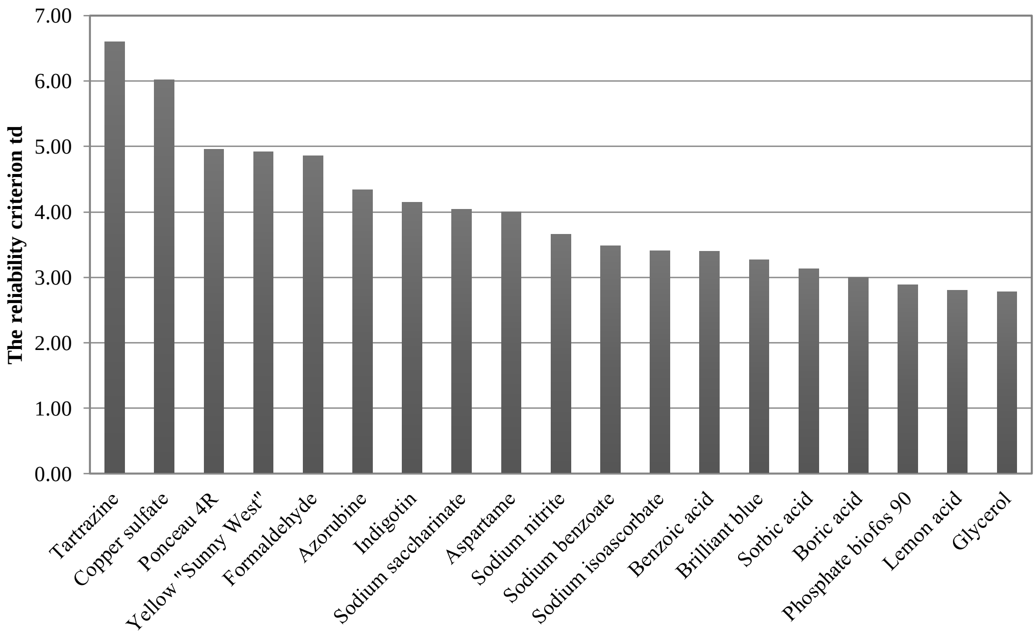

Fig.2. Value of the criterion of reliability t_d for the studied food additives

As a result of determining the chronic toxic effect of the studied food additives on the test object, it has been established that the mean arithmetic survival ranged from 9.41 (for brilliant blue E133) to 11.32 (for glycerol E422). The standard deviation of survival and fecundity ranged from 0.17 (copper sulphateE519) to 1.5 (glycerol E422). The reliability of the results was determined using Student's *t*-test.

Studying the food additives has allowed establishing that their aqueous solutions cause a decrease in fecundity of *Daphnia* and thus have a chronic toxic effect on them [15], since the reliability criterion in all cases was td ≥ 2.78 (Student's *t*-test for the degree of freedom 4 with triple repetition of the study) (Fig.2).

Many substances are cumulative, i.e. their effects sum up, or they transform in the body from non-toxic to toxic form. The study of cumulative properties is necessary, since it all owsestablishing the possibility of accumulation of a substance in the body and predicting chronic intoxication [36,37]. So, at the next stage of research, the most frequently used combinations were tested: benzoic acid E200 + sorbic acid E210; sodium benzoate E211 + potassium sorbate E202; sodium nitrite E250 + sodium phosphate E339. It has been found that these combinations of food additives have an acute toxic effect on the test object since the percentage of dead *Daphnia* in all cases was more than 50% (Fig. 3).

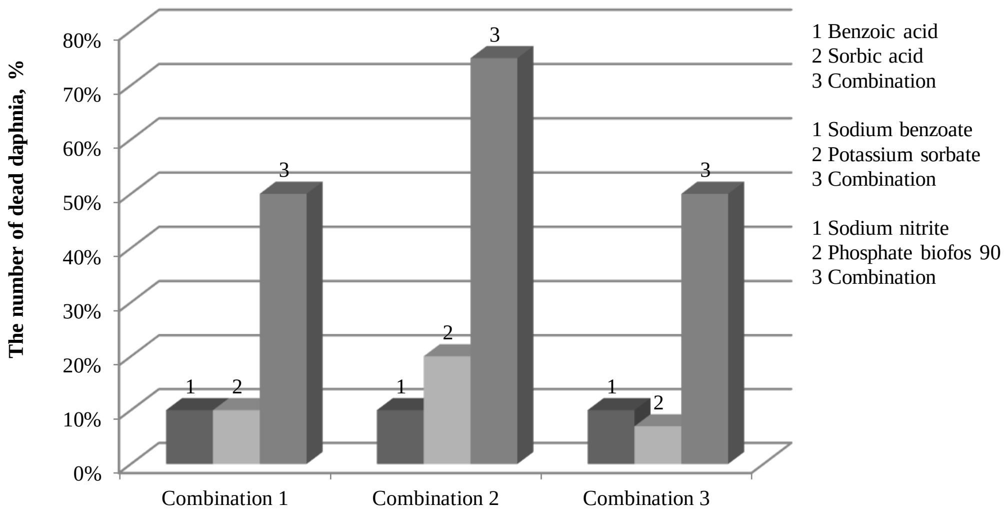

Fig.3. Results of bioassay of the combinations and components of the food additives

Conclusion

The results of the original studies allow us to conclude that the analysed samples do not have an acute toxic effect. On the other hand, it can be noted that the additives from the category of dyes are the most harmful, since all the additives studied resulted in the lethality of model organisms at the level 20–25%. Among preservatives, formaldehyde (20%) and copper sulphate (25%) caused a similar level of lethality in *Daphnia*. The 20% mortality of the model organisms was caused by the action of an aqueous solution of sodium isoascorbate (antioxidant) and saccharin (sweetener). The toxicity of such additives from the categories of stabilisers, emulsifiers, and sweeteners as sodium phosphate, glycerol, and guar gum can be neglected, since the recorded mortality was 10% or lower.

Bioassay of the chronic toxic effect produced by aqueous solutions of the food additives on model organisms has established that all samples are toxic to *Daphnia* to varying degrees. At the same time, it has confirmed the harmful effects of dyes and preservatives, in particular, tartrazine, brilliant blue, and copper sulphate.

A study of the effect of combinations of certain food additives (benzoic acid E200 and sorbic acid E210, sodium benzoate E211 and potassium sorbate E202, sodium nitrite E250 and sodium phosphate E339) on aquatic organisms has shown a significant effect. The greatest lethality (over 75%) during bioassay was caused by the combination of the additives E211 and E202.

The relationships found between the concentrations of food additives and their toxicological effects should be used to development a system ensuring the environmental safety of food products at different levels: national, regional, and local. The research results indicate the need to revise the values of the maximum permissible levels of food additives towards their reduction.

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ТОКСИКОЛОГІЧНА ОЦІНКА ХАРЧОВИХ ДОБАВОК МЕТОДОМ БІОТЕСТУВАННЯ

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Анотація. Стаття присвячена вивченню рівнів токсичності харчових добавок шляхом біотестування з використанням модельних організмів *Daphniamagna*. Охарактеризовано напрямки та методику досліджень. Проведено визначення токсичності більшості харчових добавок для максимально допустимих концентрацій. Біотестуванню піддавались харчові добавки широкого вжитку: тартразин (Е-102), жовтий «sunset» (Е-110), азорубін (Е-122), понсо 4R (Е-124), індігокармін (Е-132), діамантовий синій (Е-133), сорбінова кислота (Е-200), сорбат калію (Е-202), бензойна кислота (Е-210), бензоат натрію (Е-211), формальдегід (Е-240), нітрит натрію (Е-250), борна кислота (Е-284), ізоаскорбат натрію (Е-316), лимонна кислота (Е-330), фосфат біофос 90 (Е-339), гуарова камедь (Е-412), гліцерин (Е-422), сульфат міді (Е-519), аспартам (Е-951), сахарин (Е-954). Запропоновано скористатись концентраціями, наявними у продуктах харчування для біотестування добавок SodiumIsoascorbate E-316, Citricacid E-330, Guargum E-412, Glycerol E-422, максимальні концентрації яких не прописані у Санітарних правилах і нормах. У статті визначено гострий та хронічний токсичний вплив кожної розглянутої харчової добавки на *Daphniamagna*. Для тартразину Е-102, жовтого «sunset» Е-110, понсо 4R Е-124 та сульфату міді Е-519 гострого токсичного впливу не зафіксовано, оскільки летальність модельних організмів не перевищувала 25%. Виявлений синергетичний ефект від використання популярних комбінацій харчових добавок у різноманітних продуктах, а саме: бензойної кислоти Е-200, тасорбінової кислоти Е-210, бензоату натрію Е-211 та сорбату калію Е-202, нітриту натрію Е-250 та фосфату натрію Е-339. Саме комбінація добавок Е-211 та Е-202 спричинила найбільшу летальність (понад 75%) під час біотестування. Встановлено, що найбільша токсична дія притаманна для всіх досліджених комбінацій харчових добавок порівняно з токсичним впливом окремих добавок. Запропоновано продовжити дослідження у даному напрямку. Результати досліджень зумовлюють необхідність зменшення чинних максимально допустимих рівнів харчових добавок.

Ключові слова: харчова добавка, токсичність, біотестування, тест-об'єкт, летальність.