ORIGINAL ARTICLE

HYGIENIC REGULATIONS FOR THE SAFE APPLICATION OF COMBINED PESTICIDES IN THE CEREAL CROPS' CHEMICAL PROTECTION SYSTEM

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ABSTRACT

The aim: Hazard characterization and assessment of combined exposure to several pesticides when they simultaneously get to the human body with bakery products.

Materials and methods: Methods of analytical analysis of the range of pesticide active substances, which are registered and used in modern systems of grain crop protection in Ukraine, were used in the study. Normative documents of national legislation on hygienic regulation of pesticides and methodological approaches to assessing the combined effects of pesticide mixtures in food products serve as materials for assessment.

Results: It is established that the total risk of exposure to residual amounts of pesticides in bread products (wheat and rye bread) during its comparable getting into the body was 0.59 for children 2-6 years and 0.36 - for adults, with an allowable value of ≤ 1.0. The combined effect of pesticides per unit of children's body weight is higher, but also lies within acceptable limits. The largest contribution to the overall risk of combined exposure to triazoles is made by flutriafol (38.5-47.0%), which in the future may be the basis for substantiating measures to reduce such exposure and make appropriate management decisions.

Conclusions: Safety of consumption of agricultural products is ensured by strict following hygienic regulations for the pesticide application (application rates, frequency of treatments, pre-harvest intervals), which makes it impossible to accumulate their residual amounts in food. Triazole pesticides, widely used in almost all crop protection systems, pose a potential risk of adverse health effects due to additive or synergistic effects.

KEY WORDS: pesticides, maximum residue levels, combined impact, health risk

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INTRODUCTION

Agriculture in Ukraine is one of the main sectors of the economy, providing more than 10 per cent of the gross domestic product (GDP). The positive balance of foreign trade in agricultural goods in recent years has reached more than 13.5 billion US dollars and accounts for 40% of the total exports of Ukraine [1, 2]. A prominent place in modern agricultural production and general, the volume of exports is occupied by cereals (wheat, barley); crop areas in Ukraine as of 2019 amounted to 9424.9 thousand hectares [3]. Much attention is paid to modernization to increase crop yield production, accurate technologies in sowing and processing, modern plant protection systems, and new, resistant to diseases and weather conditions hybrids of seed. All this contributes to more efficient management than just the accumulation of land resources [4].

Chemical plant protection products remain the leading factor in increasing agricultural production. Agricultural holdings, farms, and agricultural cooperatives need 38-40 thousand tons of pesticides annually to conduct an efficient process of growing agricultural products [5]. In order to enhance biological action and prevent the development of resistance mechanisms, there is a need to find new active substances. In recent years, the trend of increasing the number of complex mixtures of pesticide formulations containing two or more active substances, the toxic properties of which may be summed up (additive effect) or enhanced (synergistic effect) with a combined impact on man [6-8].

Legal regulation of pesticide used in Ukraine is provided by the existing legal framework. All new preparative forms of pesticides are undergoing state tests for their intended purpose and further use in agricultural production of Ukraine. The criteria for the safety of pesticides for human health are the hygienic regulation of their content in the human environment.

The main standard that minimizes the harmful effects of pesticides on the human body when consuming food is maximum residue level (MRL) - the maximum level of pesticide residues in food, recog-

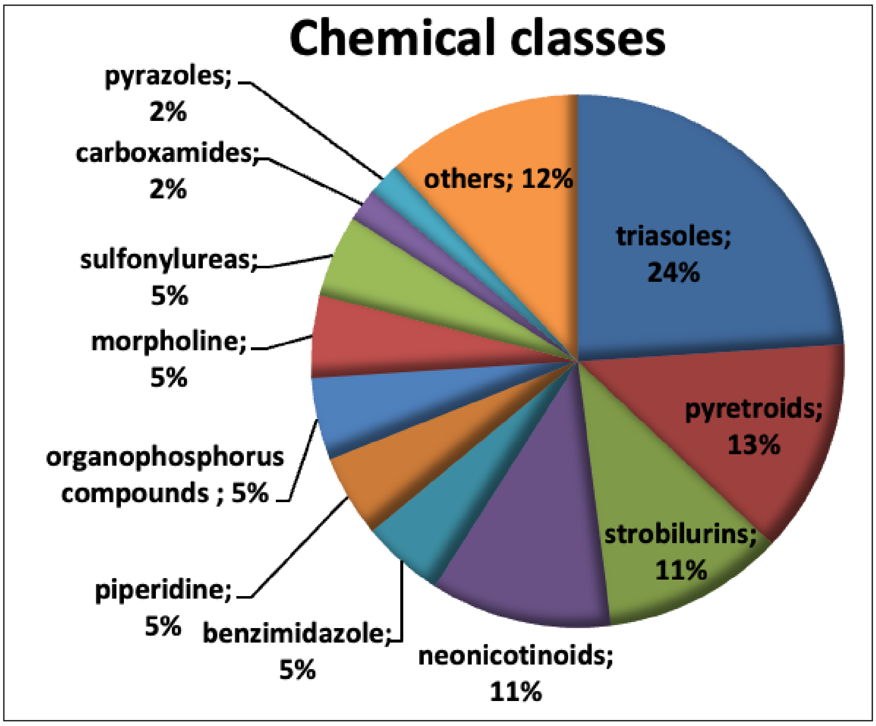

Fig. 1. Distribution of active substances of pesticides by chemical classes
(triazole – 24%, pyrethroid – 13%, strobilurin – 11%, neonicotinoids – 11%, benzimidazole – 5%, piperidine – 5%, organophosphorus compounds – 5%, morpholine – 5%, sulfonylurea – 5%; carboxamides – 2%, pyrazole – 2%, other – 12%)

nized by law as acceptable if properly use of pesticides in accordance with the principles of good agricultural practice. This level of residue should be as low as possible by modern analytical methods and be safe for the health of consumers.

However, a legally established MRL at the state level only applies to one active pesticide substance. It precludes the assessment of the additive or synergistic effect of several pesticides on the human body when they are detected together, even in small concentrations and within established standards. The presence of residues of several pesticides in food products encourages the development of methodological approaches to assessing their cumulative effects and is an important issue in the hygienic regulation of pesticides.

The importance of conducting research to develop a methodology for assessing the cumulative and synergistic effects of pesticides is stated in EU Regulation № 396/2005 of the European Parliament and of the Council on maximum pesticide residue levels [9]. There is a need to consider the cumulative effects of pesticides when setting MRLs when methods become available to assess such impacts.

Currently, there are no scientifically sound approaches to regulating the simultaneous presence of several pesticides in the same food product and clearly defined risk assessment methods that consider all multicomponent mixtures' interactions.

Addressing the combined effects of chemicals, including pesticides, on human health is one of the key initiatives of the EU Chemicals Strategy, adopted by the European Commission on 14 October 2020 under the European Green Course to achieve a non-toxic environment. It is for pesticides that progress has been made in developing a targeted methodology for a better and more accurate way to assess cumulative risk [10].

On the example of the range of active substances of combined preparations used in the system of chemical protection of cereals in Ukraine, we assessed the risk of combined effects of pesticides on human health when they come with bakery products.

THE AIM

The purpose of the research is hazard characterization and assessment of combined exposure to several pesticides when they co-enter the human body with bakery products.

MATERIALS AND METHODS

The objects of the study were the active substances of combined pesticides for various functional purposes (insecticides, fungicides, herbicides) used in Ukraine to protect cereals for various functional purposes [11], and their combined effects on human health when getting into the body with bakery products. The range of pesticides was divided into classes of chemical compounds, determining the nature of toxic effects and dose levels at which no damaging

Table I. Formulas used for risk assessment

Formula	Definition
$E_i = \frac{I_i \times MRL}{M}$	E_i – daily exposure to pesticide residues in bread products, mg / kg bw / day. MRL – maximum residue level of pesticide in cereal grains, mg / kg; I_i – average daily consumption of bakery products (wheat and rye bread), kg / person / day (for an adult - 0.28 kg, children from 2 to 6 years - 0.12 kg) [13]; M – body weight of an adult (60 kg) and children aged 2-6 years (15.6 kg) [14].
$HQ_i = \frac{E_i}{ADI_i}$	HQ_i – hazard quotient for each pesticide active substance, which characterizes the risk associated with the intake of a particular pesticide active substance with bakery products; ADI – acceptable daily intake of the active substance of the pesticide for humans, which characterizes the risk associated with the intake of a pesticide with bakery products; ADI – acceptable daily intake of the pesticide for humans, mg / kg bw / day.
$HI = \sum HQ_i$	HI – hazard index, which is the sum of the hazard quotient of each active substance and characterizes the risk of potential adverse effects from the combined exposure to several active substances when they co-enter the human body with bread products.
$PRF_i = \frac{NOAEL_{ic}}{NOAEL_i}$	RPF_i – the relative potential factor of each active substance from the group of related; $NOAEL_{ic}$ – no observed adverse effect level (dose level of the index compound at which no damaging effects are observed), mg / kg bw; $NOAEL_i$ – no observed adverse effect level (dose level of an individual compound at which no damaging effects are observed), mg / kg bw.
$E_{total} = \sum_{i=1}^{ni} \frac{I_i \times MRL_i}{M} \times PRF_i = \sum_{i=1}^{ni} E_i \times PRF_i$	E_{total} – the total exposure, the combined effect, is estimated by summing the daily exposure of each substance (E_i) multiplied by the corresponding RPF_i.

Table II. Estimation of the combined exposure to active substances of pesticides at their joint intake by organism with bakery products

Active substance	Chemical class*	ADI, mg/kg bw/d	MRL, mg/kg product	E_i, mg/kg bw/d		HQ_i	
				children, 2-6 year	adults	children, 2-6 year	adults
Alpha-cypermethrin	P	0,005	0,01	0,00008	0,00005	0,016	0,010
Azoxystrobin	S	0,03	0,2	0,00154	0,00093	0,051	0,031
Epoxiconazole	T	0,004	0,05	0,00038	0,00023	0,095	0,058
Imidacloprid	N	0,06	0,01	0,00080	0,00050	0,013	0,005
Lambda-cyhalothrin	P	0,003	0,01	0,00008	0,00005	0,027	0,017
Pyraclostrobin	S	0,02	0,2	0,00154	0,00093	0,051	0,031
Propiconazole	T	0,04	0,04	0,00077	0,00047	0,019	0,012
Tebuconazole	T	0,03	0,2	0,00154	0,00093	0,051	0,031
Flutriafol	T	0,01	0,1	0,00077	0,00047	0,077	0,047
Cyproconazole	T	0,002	0,05	0,00038	0,00023	0,190	0,115
			Total risk HI:			0,59	0,36

Notes: * P – Pyrethroids; S – Strobilurins; T – Triazoles; N – Neonicotinoids

effects (NOAEL). Theoretical research of normative documents of national legislation was conducted to find reference values for active substances of pesticides and other standards: acceptable daily intake (ADI), maximum residue levels (MRL) in cereals [12], average daily norms (ADN) of bread consumption and rye) [13]. The study also included reports from the European Food Safety Authority (EFSA) and other scientific publications on the toxicological effects of the studied pesticides.

The subject of the study was the exposure levels of pesticide active substances in cereals at the level of MRL and the total potential risk of their combined impact on the health of people of different ages (adults and children aged 2-6 years).

Risk characterization and assessment of combined exposure to pesticides were performed using the formulas listed in Table I.

The estimated total risk of exposure expresses the overall hazard according to the equivalent exposure of the index compound, and therefore can be compared with the ADI of the index compound [16].

Exposures at the level of MRL pesticides in cereals without taking into account the processing factors of agricultural raw materials, which depend on the chemical characteristics of the pesticide, product

Table III. NOAELs of triazoles by hepatic toxicity

Active substance	NOAEL, mg/kg bw/d	Effects	References
Epoxiconazole	0,8 (18-month, mouse)	Hepatotoxicity: increased body liver weight, clinical chemistry, histology, liver cell adenomas and carcinomas in mice	EFSA, 2008 (https://doi.org/10.2903/j.efsa.2008.138r
Propiconazole	3,6 (2-year rat study)	Hepatotoxicity (hypertrophy of hepatocytes, disorders of carbohydrate and fat metabolism)	EFSA, 2017 (https://doi.org/10.2903/j.efsa.2017.4887)
Tebuconazole	3,0 (1-year dog study)	Liver toxicity (rat and mouse), inducer of monooxygenase system, anemia, pathological changes in the adrenal glands, liver tumors in mice of sensitive lines, changes in the lens of the eye (dogs)	EFSA, 2014 (https://doi:10.2903/j.efsa.2014.3485)
Flutriafol	1,0 (2-year rat study)	Liver toxicity (rat and mouse), increased liver weight and histopathology, inducer of monooxygenase system, anemia	EFSA 2010 https://doi.org/10.2903/j.efsa.2010.1868
Cyproconazole	1,0 (2-year rat study); 1,84 (18-month, mouse)	Main target organ of cyproconazole is the liver upon short-term to long-term exposure (increased relative liver weight, increased incidence of hepatocellular hypertrophy, hepatocellular adenomas); probable carcinogen for humans, selective effect on reproductive function	EFSA 2010 https://doi.org/10.2903/j.efsa.2010.1897

Table IV. Relative potential factors and overall risk of combined exposure to triazole pesticides

Active substance	NOAEL, mg/kg bw/d	ADI, mg/kg bw/d	PRF	E_i, mg cyproconazole eq./kg bw/d		% ADI_{IC}	
				children, 2-6 year	adults	children, 2-6 year	adults
Epoxiconazole	0,8	0,004	1,25	0,00048	0,00029		
Propiconazole	3,6	0,04	0,28	0,00022	0,00013		
Tebuconazole	3,0	0,03	0,33	0,00051	0,00031		
Flutriafol	1,0	0,01	1,0	0,00077	0,00047		
Cyproconazole **(IC)**	1,0	0,002	1,0	0,00038	0,00023		
Total exposure, E_{total}:				0,002	0,001	100,0	50,0

and type of treatment, were used to assess the risk of combined pesticide exposure. For example, the baking of bread process can reduce pesticide residues in the final product, thus reducing the potential adverse effects on humans. Unfortunately, the lack of available information on the distribution of residues between agricultural raw materials and the finished product did not allow us to take into account this processing factor in the risk assessment.

RESULTS

The range of pesticides registered in Ukraine on cereals includes a significant number of mixed preparations of different directions of action, including 5 - herbicides, 33 insecticides and 84 - fungicides. They contain 45 active substances pertaining to various classes of chemical compounds (Fig 1).

The largest number of combinations of active substances in mixed preparations occurs with tebuconazole (27), propiconazole (20), cyproconazole (21), azoxystrobin (17), epoxyconazole (17), epoxiconazole (15), imidacloprid (15), lambda-cyhalothrin (12), flutriafol), alpha-cypermethrin (5), pyraclostrobin (5). This increases the likelihood of their combined use in an integrated cereal protection system and, at the same time, the possibility of their combined effects on human health. The above pesticides were selected to characterize the hazards and assess the combined exposure when they co-enter the human body with bread products (Table II)

The calculated hazard ratios of pesticide residues in bread products for each individual pesticide and in total do not exceed 1.0, which characterizes the impact as acceptable for different age groups.

Further research was aimed at assessing the cumulative effects of substances that are characterized by a similar method/mechanism of biological action and affect the same organ system. Such substances include pesticides from the class of triazoles, grouped in a cumulative assessment group (CAG) for their ability to have hepatotoxic effects [15]. This group includes epoxiconazole, propiconazole, tebuconazole, flutriafol, cyproconazole, which have a general toxic effect on the body with a predominant hepatotropic effect, and are inducers of the monooxygenase system. The main critical effects of their toxic action and dose levels at which observed to have no damaging effects (NOAEL) are shown in Table III.

To assess the overall total hazard of triazole exposure, we used the concept of relative potential factor (PRF). This indicator is used to determine the potential toxicity of a mixture of substances characterized by a similar mode of action. For this purpose, one pesticide from the group of related was selected as an index compound with a certain RPF, and the RPF of other substances was calculated relative to the index compound. Cyproconazole was selected as the index compound, which is characterized by the most complete toxicological database, including the effect on the liver. For the index compound, PRFs for other substances were calculated (Table IV).

As can be seen from the data in Table 4, according to the equivalent of cyproconazole, the total exposure of residual amounts of triazoles in the consumption of bread products of different age groups is 0.002 mg/kg for children (2-6 years) and 0.001 mg/kg for adults and does not exceed the reference value of the index compound. It was found that the combined effect of pesticides per body weight unit of children is higher, but also lies within acceptable limits.

The contribution of pesticides to the overall total risk of combined exposure to triazoles was ranked: flutriafol - (38.5-47.0)%; tebuconazole - (25.5-31.0)%; epoxiconazole - (24.0-29.0)%; cyproconazole - (19.0-23.0)%; propiconazole - (11.0-13.0)%. The largest contribution to the overall exposure is made by flutriafol.

DISCUSSION

Bakery products are the basic foodstuffs across the globe and the quality of such products includes safety parameters. Safety of bakery products is impossible without control of the grain used for its production. While organic bread and bakery products, like other organic food products, has become more and more popular in recent decades, it still makes minor contribution in general food intake. In terms of organic bread, Germany having the largest market share in Europe, just makes approximately only 8.5 percent of its bread market being made up of organic bread. Germany is followed by France and Italy with the next biggest markets for organic bread [17]. But other bakery products are made from grain grown with pesticide application.

Usually, foods can contain only those pesticides that have been used to protect crops. Therefore, the probability of additive effects of pesticides with different mechanisms of biological action (eg, herbicide, fungicide, insecticide) is quite low. Increased additive action can occur in the case of exposure to one pesticide by different types of exposure (water, food); when using different foods containing residues of the same pesticide; summation of toxic effects when eating foods containing different pesticides, characterized by the same type of biological action [18].

As we found cereal crops in Ukraine are treated with particular types of pesticides. In particular fungicides have leading place increasing the likelihood of their combined use in an integrated cereal protection system and, at the same time, the possibility of their combined effects on human health.

We calculated hazard ratios of pesticide residues in bread products for each individual pesticide in total and found that its do not exceed 1.0, concluding that the impact is acceptable for different age groups.

It was found that among studied fungicides flutriafol topped with the (38.5-47.0) % of the overall total risk of combined exposure, which in the future can be the basis for justifying measures to reduce such impact and make appropriate management decisions.

CONCLUSIONS

1. Research has been conducted to assess the risk to consumer health from the combined effects of pesticides used in cereals. Findings indicate that the total risk of exposure to residual active substances of pesticides in bread products (wheat and rye bread), when combined getting into the body, was 0.59 for children 2-6 years and 0.36 - for adults with a permissible value ≤ 1,0.
2. The conclusion about the acceptable level of total risk for the health of consumers at strict following the hygienic regulations of pesticides application (application rates, frequency of treatments, pre-harvest intervals), as precautionary measures to prevent accumulation of residual quantities of pesticides active substances in foodstuff.
3. Despite the acceptable risk to the population when ingested with bread products, the potential danger of exposure to triazoles should be of concern due to their widespread use on all crops and the possibility of increased adverse health effects through additive or synergistic effects.

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