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ACUTE TOXICITY ESTIMATION OF MULTICOMPONENT PLANT PROTECTION PRODUCTS USING CALCULATIONS, IN SILICO AND IN VIVO METHODS. PERSPECTIVES FOR UPDATING APPROACHES TO CLASSIFICATION AND RISK ASSESSMENT

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Introduction. Plant protection products (PPP) are used extensively globally and in Ukraine to save plants from pests and diseases causing yield loss and foodstuff deterioration. Despite its importance for crops protection, plant protection products contain active ingredients (pesticides) and co-formulants that are potentially poisonous not only for target species (pests and diseases of plants) but may also lead to acute and chronic intoxications of other, not target species, including humans [1,2]. Currently many plant protection products are complex mixtures of few active ingredients and other ingredients, here called co-formulants. For example, in the State register of pesticides and agrochemicals authorized for use in Ukraine, among 3815 plant protection products authorised for the moment and having registration ending in 2021 and later, about one thousand products have 2 or more active ingredients [3].

In most countries (including Ukraine) PPP are strictly regulated and number of toxicity studies on active ingredient(s) and formulation itself have to be performed before placing product to the market, including study of acute oral toxicity and determination of LD_{50} [4,5]. LD_{50} for laboratory animals (usually rats) is used not only for risk assessment of substances (e.g. for calculation of acute reference doses), but also for classification and labelling of a chemical substances and products. There is Globally Harmonized System of Classification and Labelling (GHS) exists and is adopted by all developed countries (72 countries in total). In EU mentioned document is implemented as Regulation on classification, labelling and packaging of substances and mixtures (CLP) [6]. In Ukraine, despite work is ongoing now on implementation of GHS in Ukraine by approximation of Ukrainian legislation with EU, namely with Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures(CLP) it has not been implemented yet in Ukraine [7]. For PPPs Hygienic Classification of Pesticides by the Degree of Hazard currently is in force in Ukraine [8].

Table 1 – Difference in GHS and Hygienic classification of pesticides by acute oral toxicity

	Hazard categories of acute toxicity class "Acute toxicity" according to GHS				
	Category 1	Category 2	Category 3	Category 4	Category 5 (not used in EU)
LD_{50} (or ATE) (mg/kg of body weight)	≤ 5	5-50	50-300	300-2000	2000-5000
LD_{50} (mg/kg of body weight) for solid formulations	≤15	15-50	51-500	≥ 500	
LD_{50} (mg/kg of body weight) for liquid formulations	≤50	50-200	201-2000	≥ 2000	
	1	2	3	4	
	Hazard classes of acute oral toxicity according to Hygienic Classification of Pesticides by the Degree of Hazard				

Among other differences of the mentioned classifications **(see table 1),** GHS offers to use calculated acute toxicity estimate for classification of mixtures, which is not foreseen in Ukrainian classification. This approach uses a formula (often mentioned in the literature as "GHS additivity formula") presented on the **fig.**

Despite the fact that use of the GHS formula theoretically may replace animal use and considerably reduce costs in the assessment of mixtures, its use is still discussed. One of the main reasons for doubts is that formula not accounts for interaction of components, both toxicokinetic and toxicodynamic [9-15].

Another option for replacement (or at least reduction) of animal use in the acute toxicity assessment is the use of in silico or computational tools

(including quantitative structure-activity relationships (QSAR) models, decision trees, rule based models). There are number of published studies and reviews assessing currently available tools [16-18].

In this work, we combined all possible approaches for mixture acute toxicity assessment of PPP containing more than one active ingredient (i.e. in vivo studies as reference point, calculation method and in silico modelling).

Aim of study. Aim of this study is to assess different alternative approaches to acute toxicity assessment of PPP, including calculation based on the assumption of additivity and in silico methods.

Object and methods of the study. In this study we assessed acute toxicity of eight PPP, containing from 2 to 5 active ingredients (AI) pesticides and number of co-formulants. In the **table 2** relevant data on the active ingredients and its content in the studied PPP is presented. For further calculations, worst case scenario approach was used and where two data-points for LD_{50} are available for single AI (i.e. for male and female rats) we used smaller one.

We conducted studies of acute toxicity (Wistar Han rats, OECD 425 [19]) of eight pesticide formulations, containing from 2 to 5 pesticides (azoxystrobin, cyproconazole, difenoconazole, flutriafol, imidacloprid, lambda-cyhalothrin, propiconazole, spiroxamine, tebuconazole, thiabendazole, thiram, triadimefon, triadimenol).

The animals were obtained from the breeding vivary of small animals of the State Enterprise «L. I. Medved's ResearchCenter of Preventive Toxicology, Food and Chemical Safety of the Ministry of Health of Ukraine». Experimental animals were quarantined for 5 days. Animals were kept indoors at a temperature of 22 ± 3 ° C, relative humidity – 40-60%. The diet of animals – concentrated granular feed produced by Altromin (Germany). Animals received disinfected by ultraviolet irradiated and filtered using reverse osmosis water (without restrictions).

The in vivo studies were conducted in accordance with the requirements of the principles of GLP (Good Laboratory Practice) set out in OECD guidelines and Directive 2004/10/EC [20,21]. Furthermore, requirements and provisions of the «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» and "Guide for the Care and Use of Laboratory Animals" were used to ensure animal welfare [22,23].

To derive in silico prediction of LD_{50} of active ingredients we used EPA Toxicity Evaluation Software Tool (T.E.S.T.) [24]. Module that predicts LD_{50} in the T.E.S.T. is based on oral rat LD_{50} dataset contained 7420 chemicals obtained from the ChemIDplus database. Predictions are derived with a help of five QSAR methods, namely hierarchical method, FDA method, Single-model method, Group contribution method and Nearest neighbour method. In addition, T.E.S.T. takes advantage of all mentioned above methods of prediction using the consensus method. This latter approach takes into account applicability domain of the predicted LD_{50} from the above mentioned methods and calculates consensus prediction. This method provides more accurate estimations, as erroneous predictions are extinguished by predictions of other methods (18). This method

$$ATE_{mixture} = \frac{100}{\sum_n \frac{C_i}{ATE_i}}$$

$ATE_{mixture}$ is Acute Toxicity Estimate of mixture (e.g. LD_{50});
Ci – concentration of component in mixture, %;
ATE_i – Acute Toxicity Estimate (LD_{50}) of ingredient;
n –number of ingredients.

Figure – GHS additivity formula.

was used to derive LD_{50} in silico predictions of PPP active ingredients studied in this work.

Then, we calculated Acute Toxicity Estimate for mixture (ATEmix), using GHS additivity formula **(fig.),** taking into account in vivo LD_{50} of only active ingredients of PPP, of all ingredients of formulation and LD_{50} of active ingredients predicted by T.E.S.T. [25].

Then, we attributed PPPs to relevant hazard category of GHS and Hygienic classification based on in vivo study of formulation and on calculations of ATE (based on the active ingredients only, on all ingredients and using predicted by T.E.S.T. values of LD_{50}). Additionally, we calculated additivity coefficient (by dividing experimental and calculated values of ATE for mixture based on all ingredients).

Results and discussion. Results of consensus method modelling of rat oral LD_{50} for pesticide active ingredients of PPP studied in this work performed using T.E.S.T. and LD_{50} determined in vivo are presented together with percent of deviation in **table 3.** Quite high deviation of predicted LD_{50} values from experimental ones may be explained by small sample size in this study (i.e. only 13 compounds). In the larger scale study with performance of T.E.S.T on the 7417 compounds in terms of regression (coefficient of determination – r2) between experimental data and the predictions expressed in log units as mg/kg,

Table 2 – Multicomponent PPP studied in this work

PPP	Active ingredients (AI)	Content of AI, g/l	LD_{50} of AI, mg/kg (rats)
No. 1	Tebuconazole	125	2330 ♂ / 1260 ♀
	Triadimefon	100	569 ♂, ♀
No. 2	Azoxystrobin	200	5000 ♂, ♀
	Cyproconazole	80	1020 ♂ / 1330 ♀
No. 3	Propiconazole	125	1517 ♀
	Azoxystrobin	100	5000 ♀
	Cyproconazole	30	1333 ♀
No. 4	Tebuconazole	167	4000 ♂ / 1700 ♀
	Triadimenol	43	721 ♂, ♀
	Spiroxamine	250	595 ♂ / 560 ♀
No. 5	Imidacloprid	300	450 ♀
	Lambda-cyhalothrin	100	64 ♂, ♀
No. 6	Imidacloprid	280	681 ♂, ♀
	Thiabendazole	80	3100 ♂, ♀
No. 7	Spiroxamine	300	595 ♂ / 560 ♀
	Tebuconazole	120	4000 ♂ / 1700 ♀
	Difenoconazole	30	1453 ♂,♀
No. 8	Imidacloprid	160	430 ♂ / 422 ♀
	Lambda-cyhalothrin	25	87,5 ♂ / 105 ♀
	Flutriafol	30	1260 ♂, ♀
	Thiram	100	3700 ♂ / 1800 ♀
	Tebuconazole	7	2330 ♂ / 1260 ♀

Table 3 – Results of prediction of oral rat LD_{50} by EPA T.E.S.T and its deviation from experimentally determined LD_{50}

Active ingredient	LD_{50} experimental, mg/kg body weight	LD_{50} predicted by T.E.S.T., mg/kg body weight	Deviation from experimental, %
Azoxystrobin	5000	688,92	-86,2
Cyproconazole	1020 ♂ / 1330 ♀	1549,34	51,9 ♂ / 16,5♀
Difenoconazole	1453	943,48	-35
Flutriafol	1260	681,05	-46
Imidaclopride	450	369,01	-18
lambda-Cyhalothrine	64	442,4	591,3
Propiconazole	1517	1026,26	-32,3
Spiroxamine	595 ♂ / 560 ♀	2765	364,7 ♂ / 393,7♀
Tebuconazole	4000 ♂ / 1700 ♀	2131,75	-46,7 ♂ / 25,4♀
Thiabendazol	3100	472,52	-84,8
Thiram	3700 ♂ / 1800 ♀	1525,03	-58,8 ♂ / -15,3♀
Triadimefon	363	847,06	133,3
Triadimenol	3801,88	1530,41	-59,7
Mean deviation of predicted LD_{50} values from experimental			62,95%

Table 4 – Results of determination of LD_{50} in vivo performed for mixtures (PPPs) and of ATE calculation using GHS additivity formula

PPP	Rat LD_{50} of PPP determined *in vivo,* mg/kg b.w.	Data on calculations based on AI only		Data on calculations based on all ingredients		Data on calculations based on LD_{50} predicted by T.E.S.T.	
		ATE, mg/kg b.w.	Deviation, %	ATE, mg/kg b.w.	Deviation, %	ATE, mg/kg b.w.	Deviation, %
No. 1	2546	3641	43,0	2317	-9,0	3015	18,4
No. 2	5000	8450	69,0	6850	37,0	2703	-45,9
No. 3	2000	7620	281,0	3120	56,0	2049	2,5
No. 4	625	1653	164,5	1189	90,2	2293	266,9
No. 5	310	450	45,2	440	41,9	925	198,4
No. 6	3500	2155	-38,4	1960	-44,0	1977	-43,5
No. 7	2100	1596	-24,0	945	-55,0	1605	-23,6
No. 8	900	1404	56,0	1368	52,0	1603	78,1
Mean deviation of calculated ATE from in vivo LD_{50} for mixture			74,5		21,1		56,4

and the standard deviation of prediction errors were 0,68 and 0,51 respectively [18].

Results of determination of LD_{50} *in vivo* performed for mixtures (PPPs) and of ATE calculation using GHS additivity formula taking into account only AI, all ingredients and using LD_{50} values predicted by T.E.S.T. for active ingredients are presented in the **table 4.** As one can see, most accurate results of ATE calculation as compared to in vivo data are obtained when all ingredients of formulation are taken into account (mean deviation – 21,1%), surprisingly followed by results based on LD_{50} predicted by in silico tool. In the **table 5** correlation quotients between datasets of in vivo LD_{50} determination and calculated using GHS additivity formula for studied in this work PPP are presented.

Of the 8 studied in this work PPPs, results of calculations using GHS additivity formula lead to misclassification according to GHS of 2 formulations (75% correct classification) when only active ingredients LD_{50} in vivo data was used; 3 formulation were misclassified (62.5% correct classification) when only active ingredients LD_{50} in vivo data was used; classification based on prediction of LD_{50} with in silico tool in this study was not better than tossing a coin (i.e. only 50% of correct classifications). Underestimation of hazard category was observed for one PPP when calculation were made on the basis of in vivo data and for 2 formulations when we used predictions from T.E.S.T as input. Detailed information regarding performance of approaches tested in this work presented in **table 6.**

Results of classification exercise based on in vivo data and calculated values according to Ukrainian Hygienic Classification of Pesticides by the Degree of Hazard is presented in **table 7.** In this situation misclassification was observed in 3 cases (37,5%) when calculation was made on the basis of AI only and using in silico predictions. Surprisingly, performance of GHS additivity formula taking into account all ingredients of formulation was lower – only half PPPs were correctly classified.

Main cause of inaccurate acute toxicity estimation upon GHS additivity formula use and resulted misclassification lies in its inherent assumption of additivity of toxicity of all ingredients in the mixture. Despite such assumption may be appropriate for some mixtures, it is obvious that for vast number of the mixtures, including many of PPP, it is not a case. Interactions may take place on chemical level in the mixture with following changes in toxicokinetic and toxicodynamic profiles [26-29]. In this work we have not assessed with scrutiny possible ways of interactions of studied mixtures, however we tried to assess it quantitatively using additivity coefficient, which is result of ATE calculation devided by experimental LD_{50}. When assessing data of the additivity coefficients, **(see table 8)** we agreed to use as evident for presence of considerable interaction values of the coefficient less than 0,6 as a marker of antagonism (marked "---" in the table) and more than 1,5 for synergism (marked "+++" in the table). Values between 0,6 and 1,5 considered as weak interaction (marked "++" or "--" in the table), except values between 0,8 and 1,2 considered as absence of interaction (marked "=").

Additional contribution to inaccurate prediction of GHS additivity formula may be use of acute Oral Toxic Class (ATC) protocol (OECD TG no. 423) which generates results as ranges, but not discrete values. In our case it

Table 5 – Correlation coefficients between experimental and calculated acute toxicity for mixtures

Correlation between *in vivo* LD_{50} for mixtures and ATE calculated using GHS additivity formula		
0,69	0,84	0,60

is applicable to calculations made taking into account all ingredients, as data on LD_{50} of co-formulants was taken from Material Safety Data Sheets which often. Thus, possible inaccurate input may lead to possible deviations from the true values.

Another issue that is worth to mention here is that classification is based on ranges **(see table 1)** and this ranges are narrower for compounds that are more toxic. In this study, we were able to assess performance of the GHS formula together with in vivo and in silico data for weakly and moderately toxic compounds and mixtures (i.e. 3-5 Categories of acute toxicity according to GHS). Therefore, it is advisable to continue studies with more mixtures representing all range of categories of acute toxicity.

Further developments for use of additivity formula may lie in the more refined approach aimed on the taking into account ingredients interaction and read-across. One of the examples of the taking into account interaction is calculation of mixture specific LD_{50} for ingredients in different mixtures (e.g. in organic solvents and in aqueous solutions) with the known in vivo LD_{50} of the mixture [11].

Conclusions

1. Differences of calculated and tested values of acute toxicity estimates for eight multicomponent PPP did not lead to their misclassification in up to 75% of cases according to GHS when based on in vivo data.

2. Differences in calculated values of acute toxicity estimates based on in silico predicted results lead to misclassification of the half of the formulations, however it may be lower if account to variability of experimental results and small number of mixtures tested here.

3. Underestimation of the hazard according to GHS classification happened only in 12,5% of the mixtures studied here.

4. Extent of coefficients of additivity of some mixtures, especially where it shows potentiation requires more attention in further studies and assessments.

5. Correct use of the GHS additivity formula can help reduce animal testing of plant protection products. However, additional effects needed to increase its predictivity.

6. In silico approach can be used internally within a company (e.g., for product design), as a tool to predict a starting dose level for further animal testing where it required.

Prospects for further research. Further studies perspectives will include assessment in the similar way as presented here of larger sample of multicomponent plant protection products and other mixtures representing wider range of acute toxicity categories (including more toxic). Collection of such data will enable in the future development of the list of mixture type-specific LD_{50} values for active ingredients (e.g. depending on solvents) and their application in the PPPs classification and risk assessment.

Table 6 – Classification of PPP according to GHS based on different approach to acute toxicity assessment

PPP	GHS category based on *in vivo* data	GHS category according to calculated ATE based on AI only	GHS category according to calculated ATE based on all ingredients	GHS category according to calculated ATE based on LD_{50} predicted by T.E.S.T.
No. 1	5	5	5	5
No. 2	5	5	5	5
No. 3	4	5	5	5
No. 4	4	4	4	5
No. 5	4	4	4	4
No. 6	5	5	4	4
No. 7	5	4	4	4
No. 8	4	4	4	4
Correctly predicted, number (%)		6/8 (75%)	5/8 (62,5%)	4/8 (50%)
Underestimated category, number (%)		1 (12,5%)	1 (12,5%)	2 (25%)

Table 7 – Classification of PPP according to Ukrainian Hygienic Classification of Pesticides by the Degree of Hazard based on different approach to acute toxicity assessment

PPP	Hazard class based on *in vivo* data	Hazard class according to calculated ATE based on AI only	Hazard class according to calculated ATE based on all ingredients	Hazard class according to calculated ATE based on LD_{50} predicted by T.E.S.T.
No. 1	4	4	4	4
No. 2	4	4	4	4
No. 3	3	4	4	4
No. 4	4	3	3	4
No. 5	3	3	3	3
No. 6	4	4	3	3
No. 7	4	3	3	3
No. 8	3	3	3	3
Correctly predicted, number (%)		5/8 (62,5%)	4/4 (50%)	5/8 (62,5%)
Underestimated hazard class, number (%)		1 (12,5%)	1 (12,5%)	1 (12,5%)

Table 8 – Coefficients of additivity (ATE calculated/LD_{50} experimental) and its interpretation

PPP	When compare with ATE calculated on the basis of AI only		When compare with ATE calculated on the basis of all ingredients		When compare with ATE calculated on the basis of LD_{50} predicted by T.E.S.T	
	Coefficients of additivity	Joint action interpretation	Coefficients of additivity	Joint action interpretation	Coefficients of additivity	Joint action interpretation
No. 1	1,43	++	0,91	=	1,18	=
No. 2	1,69	+++	1,37	++	0,54	- – -
No. 3	3,81	+++	1,56	+++	1,02	=
No. 4	2,64	+++	1,90	+++	3,67	+++
No. 5	1,45	++	1,42	++	2,98	+++
No. 6	0,62	- -	0,56	- – -	0,56	- – -
No. 7	0,76	- -	0,45	- – -	0,76	– -
No. 8	1,56	+++	1,52	+++	1,78	+++

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ОЦІНКА ГОСТРОЇ ТОКСИЧНОСТІ БАГАТОКОМПОНЕНТНИХ ЗАСОБІВ ЗАХИСТУ РОСЛИН З ВИКОРИСТАННЯМ РОЗРАХУНКІВ, МЕТОДІВ *IN SILICO* ТА *IN VIVO*. ПЕРСПЕКТИВИ ОНОВЛЕННЯ ПІДХОДІВ ДО КЛАСИФІКАЦІЇ ТА ОЦІНКИ РИЗИКІВ

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Резюме. Метою дослідження є оцінка різних альтернативних підходів до оцінки гострої токсичності засобів захисту рослин (ЗЗР), включаючи метод розрахунків, який базується на припущенні щодо адитивності та методами *in silico*. Була оцінена гостра токсичність восьми ЗЗР, що містять від 2 до 5 діючих речовин пестицидів та ряд допоміжних речовин. Дослідження гострої токсичності *in vivo* проводились згідно з OECD 425. Для отримання *in silico* прогнозів LD_{50} діючих речовин було використано програмний засіб оцінки токсичності EPA (T.E.S.T.). Розрахунок оцінки гострої токсичності для сумішей проводився із застосуванням формули адитивності GHS, беручи до уваги *in vivo* LD_{50} лише діючих речовин ЗЗР, усіх інгредієнтів формуляції та LD_{50} діючих речовин, передбачених T.E.S.T. На підставі результатів *in vivo*, розрахунків та прогнозування *in silico* ЗЗР класифікували відповідно до СГС та Української гігієнічної класифікації пестицидів. Коефіцієнти адитивності були розраховані для оцінки ступеня взаємодії. Наведено результати згаданих досліджень *in vivo*, моделювання *in silico* та розрахунків. Середнє відхилення прогнозованих T.E.S.T. LD_{50} значень від експериментальних становило 62,95%. Середнє відхилення розрахованого ATE від *in vivo* LD_{50} для сумішей становило 74,5% (на основі даних *in vivo* LD_{50} лише для AI), 21,1% (на основі даних *in vivo* LD_{50} для всіх інгредієнтів) і 56,4% для ATE, розрахованого з використанням прогнозів T.E.S.T. Коефіцієнти кореляції для згаданих розрахунків становили 0,69; 0,84 та 0,60 відповідно. Різниця розрахункових та експериментальних значеннях оцінок гострої токсичності для восьми багатокомпонентних ЗЗР не призвела до їх неправильної класифікації у 75% випадків згідно з даними GHS на основі даних *in vivo*. Відмінності в розрахункових значеннях оцінок гострої токсичності, заснованих на результатах, передбачених *in silico*, призводять до неправильної класифікації половини рецептур, однак вона може бути нижчою, якщо врахувати мінливість експериментальних результатів та малу кількість сумішей, випробуваних тут. Недооцінка категорії небезпеки за класифікацією СГС відбулось лише у 12,5% сумішей, що вивчались тут. Подальші дослідження включатимуть оцінку більшої кількості багатокомпонентних засобів захисту рослин та інших сумішей аналогічним представленому тут чином та які представляють собою ширший діапазон категорій гострої токсичності та розробку переліку специфічних для типу сумішей значень LD50 для активних інгредієнтів (наприклад, залежно від розчинників) та їх застосування в класифікації ЗЗР та оцінці ризиків.

Ключові слова: гостра токсичність, LD_{50}, засоби захисту рослин, взаємозв'язок структура-активність, класифікація, суміші.

ОЦЕНКА ОСТРОЙ ТОКСИЧНОСТИ МНОГОКОМПОНЕНТНЫХ СРЕДСТВ ЗАЩИТЫ РАСТЕНИЙ С ИСПОЛЬЗОВАНИЕМ РАСЧЕТОВ, МЕТОДОВ *IN SILICO* И *IN VIVO*. ПЕРСПЕКТИВЫ ОБНОВЛЕНИЯ ПОДХОДОВ К КЛАССИФИКАЦИИ И ОЦЕНКЕ РИСКОВ

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Резюме. Целью исследования является оценка различных альтернативных подходов к оценке острой токсичности средств защиты растений (СЗР), включая метод расчетов, основанный на предположении о аддитивности и методами in silico. Была оценена острая токсичность восьми СЗР, содержащих от 2 до 5 действующих веществ пестицидов и ряд вспомогательных веществ. Исследование острой токсичности in vivo проводились согласно OECD 425. Для получения in silico прогнозов LD_{50} действующих веществ было использовано программное средство оценки токсичности EPA (T.E.S.T.). Расчет оценки острой токсичности для смесей проводился с применением формулы аддитивности GHS, учитывая in vivo LD_{50} только действующих веществ (ДВ) СЗР, всех ингредиентов формуляции и LD_{50} ДВ, спрогнозированных T.E.S.T. На основании результатов in vivo, расчетов и прогнозирования in silico СЗР классифицировали в соответствии с СГС и украинской гигиенической классификации пестицидов. Коэффициенты аддитивности были рассчитаны для оценки степени взаимодействия. Приведены результаты упомянутых исследований in vivo, моделирование in silico и расчетов. Среднее отклонение прогнозируемых T.E.S.T. LD_{50} значений от экспериментальных составило 62,95%. Среднее отклонение рассчитанного ATE от *in vivo* LD_{50} для смесей составило 74,5% (на основе данных in vivo LD_{50} только для ДВ), 21,1% (на основе данных in vivo LD_{50} для все ингредиентов) и 56,4% для ATE, рассчитанного с использованием прогнозов T.E.S.T. Коэффициенты корреляции для упомянутых расчетов составляли 0,69; 0,84 и 0,60 соответственно. Разница расчетных и экспериментальных значений оценок острой токсичности для восьми многокомпонентных СЗР не привела к их неправильной классификации в 75% случаев по данным GHS на основе данных in vivo. Различия в расчетных значениях оценок острой токсичности, основанных на результатах, прогнозируемых *in silico*, приводят к неправильной классификации половины формуляций, однако она может быть ниже, если учесть изменчивость экспериментальных результатов и малое количество смесей, испытанных здесь. Недооценка категории опасности по классификации СГС произошла лишь в 12,5% смесей, которые изучались здесь. Дальнейшие исследования будут включать оценку большего количества многокомпонентных средств защиты растений и других смесей аналогичным, представленному здесь, образом и представляющих собой более широкий диапазон категорий острой токсичности и разработку перечня специфических для типа смесей значений LD_{50} для активных ингредиентов (например, в зависимости от растворителей) и их применение в классификации СЗР и оценке рисков.

Ключевые слова: острая токсичность, LD_{50}, средства защиты растений, взаимосвязь структура-активность, классификация, смеси.

ACUTE TOXICITY ESTIMATION OF MULTICOMPONENT PLANT PROTECTION PRODUCTS USING CALCULATIONS, *IN SILICO* AND *IN VIVO* METHODS. PERSPECTIVES FOR UPDATING APPROACHES TO CLASSIFICATION AND RISK ASSESSMENT

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Abstract. Aim of study is to assess different alternative approaches to acute toxicity assessment of PPP, including calculation based on the assumption of additivity and in silico methods. Acute toxicity of eight PPP, containing from 2 to 5 active ingredients (AI) pesticides and number of co-formulants was assessed. In vivo studies of acute toxicity were conducted according to OECD 425. To derive in silico prediction of LD_{50} of active ingredients EPA Toxicity Evaluation Software Tool (T.E.S.T.) was used. Calculation of Acute Toxicity Estimate for mixture was done using GHS additivity formula, taking into account in vivo LD_{50} of only active ingredients of PPP, of all ingredients of formulation and LD_{50} of active ingredients predicted by T.E.S.T. On the basis of in vivo results, calculations and in silico predictions PPP were classified according to GHS and Ukrainian Hygienic classification. Additivity coefficients were calculated to assess extent of interaction. Results of mentioned in vivo studies, in silico modelling and calculations are presented. Mean deviation of predicted by T.E.S.T LD_{50} values from experimental was 62,95%. Mean deviation of calculated ATE from in vivo LD_{50} for mixture was 74,5% (based on in vivo LD_{50} data for AI only), 21,1% (based on based on in vivo LD_{50} data for all ingredients) and 56,4% for ATE calculated using T.E.S.T predictions. Correlation coefficients for mentioned calculations were 0,69;0,84 and 0,60 respectively. Differences of calculated and tested values of acute toxicity estimates for eight multicomponent PPP did not lead to their misclassification in up to 75% of cases according to GHS when based on in vivo data. Differences in calculated values of acute toxicity estimates based on in silico predicted results lead to misclassification of the half of the formulations, however it may be lower if account to variability of experimental results and small number of mixtures tested here. Underestimation of the hazard according to GHS classification happened only in 12,5% of the mixtures studied here. Further studies will include assessment in the similar way as presented here of larger sample of multicomponent plant protection products and other mixtures representing wider range of acute toxicity categories and development of the list of mixture type-specific LD_{50} values for active ingredients (e.g. depending on solvents) and their application in the PPPs classification and risk assessment.

Key words: acute toxicity, LD_{50}, plant protection products, structure-activity relationship, classification, mixtures.

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НАКОПИЧЕННЯ КАДМІЮ В ЯЄЧНИКАХ ЩУРІВ ПРИ ІЗОЛЬОВАНОМУ ВВЕДЕННІ СОЛЕЙ КАДМІЮ ТА В КОМБІНАЦІЇ З ЦИТРАТАМИ ЦЕРІЮ Й ГЕРМАНІЮ

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Зв'язок публікації з плановими науково-дослідними роботами. Експериментальне дослідження виконано у рамках науково-дослідної роботи кафедри медичної біології, фармакогнозії та ботаніки ДЗ «ДМА» «Біологічні основи морфогенезу органів та тварин під впливом мікроелементів та ультрамікроелементів в експерименті» (№ державної реєстрації 0118U006635).

Вступ. Важливою та обов'язковою умовою нормального функціонування організму є стабільність його хімічного складу. Проте в сучасних умовах високого рівня забруднення навколишнього середовища важкими металами та погіршення соціально-економічних, екологічних, біологічних факторів життя відбулися суттєві зміни в елементному статусі населення, особливо в умовах промислово розвинених територій. Особливо важливий достатній вміст та баланс макро- і мікроелементів для нормального перебігу вагітності, пологів і розвитку організму плодів.

Серед хімічних речовин, що забруднюють навколишнє середовище, важкі метали та їх сполуки утворюють значну групу токсикантів, які належать до пріоритетних забруднювачів виробничого та навколишнього середовища, тому першочергове значення досліджень у цьому напрямку неодноразово відмічалось у наукових працях. Дослідниками визначено, що у мешканців сучасного мегаполісу спостерігається накопичення в організмі різних хімічних, у тому числі токсичних, елементів, серед яких значне місце займає накопичення кадмію [1].

Кадмій не є життєво необхідним хімічним елементом для організму людини, він практично відсутній в організмі новонароджених, з віком акумулюється, і до 50 років його загальний вміст може досягати 20-30 мг [2,3]. У природі кадмій присутній у ґрунті, рудах, морській воді, в атмосферу надходить у результаті вулканічних вивержень і вивільнення з рослин [4]. Кадмій є побічним продуктом плавлення цинку і свинцю, використовується в гальванізації, виготовленні нікель-кадмієвих акумуляторів, а також в якості пігменту фарб і пластику.

Кадмій надходить в організм людини через шлунково-кишковий тракт (за добу в середньому 20-50 мкг з харчовими продуктами (м'ясо, морепродукти, овочі і злаки) та 0,1 мкг з питною водою) і дихальні шляхи (0,02 мкг) [5,6]. Особливістю біологічної дії кадмію є його здатність негативно впливати на здоров'я людини при тривалому впливі низьких рівнів забруднення у зв'язку з високим коефіцієнтом біологічної кумуляції. Відомо, що надлишок кадмію інгібує синтез ДНК, білків і нуклеїнових кислот, значною мірою змінює метаболізм і функції таких ессенціальних елементів, як цинк, залізо, мідь, марганець, кальцій, селен. Недостатня кількість цих елементів, а також білків і вітамінів збільшує токсичність кадмію [2,3,6,7]. Вважають, що найважливішим механізмом токсичної дії кадмію є блокування груп SH ферментів.