

1. Introduction

Some studies have proven possible potentiating toxic effect of combined pesticides. The potentiating effect may be related to the impact of a certain substance on the enzymatic activity of another one [1].

The pesticide market deals with active substances with carcinogenic action, e.g., benthialdicarb-isopropyl and folpet, which may be used as combined preparations [2, 3]. Both pesticides have been studied according to the international requirements, being registered in many countries [4, 5]. The studies have shown carcinogenic effect of folpet in mice and benthialdicarb-isopropyl in mice and rats. Considering probable effect of both substances on enzymatic systems, participating in xenobiotics metabolism, one should expect their carcinogenic effect potentiation when combined in the preparation [6]. The *in vivo* study of both substances mutagenic effect didn't confirm any genotoxic effect. So, both substances have been referred to epigenetic carcinogens with promotor threshold action mechanism [7, 8]. Due to this, the promotor action of the substances has been studied by the average term test of multi-organ model [9].

As the defined substances express the one-purpose effect, it is particularly important to evaluate the probability of summation, synergetic or antagonizing action.

Aim of the research is to study peculiarities of histomorphological liver changes associated with combined effect of active substances with one-purpose action.

PECULIARITIES OF HISTOMORPHOLOGICAL LIVER CHANGES ASSOCIATED WITH COMBINED EFFECT OF ACTIVE SUBSTANCES WITH ONE-PURPOSE ACTION

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Abstract: Some studies have proven possible potentiating toxic effect of combined pesticides. The potentiating effect may be related to the impact of a certain substance on enzymatic activity of another one. Due to combined preparations popularity, the importance of evaluating probability of summation and synergetic or antagonizing action of the one-purpose substances becomes particularly important.

Aim of the research is to study peculiarities of histomorphological liver changes associated with combined effect of active substances with one-purpose action.

Methods. The authors have used toxicological, histochemical and statistical study methods. 45 rats have been used for the study. The liver specimens for histochemical analysis were selected immediately after dissection of the rats, from which the 5 micrometer sections were prepared, using freezing microtome. After leaving the sections in cold acetone, the histochemical reaction on determining the γ -glutamyl transpeptidase, a transformed hepatocyte marker, which forms nodules while proliferates, was conducted.

Results. The authors have established that the above-mentioned characteristics in animals which were administered the drug (benthialdicarb-isopropyl+folpet) do not differ from negative control group. General specific area of nodules per cm² and specific quantity of nodules per cm² haven't reliably changed, the control group exceeding these data by 18 % and 12 %, respectively.

Discussion. The study of the benthialdicarb-isopropyl and folpet mixture effect on animals has not revealed liver tissue proliferation of the carcinogen-transformed cells and appearance of hyperplastic nodes, which express the γ -glutamyl transpeptidase, a histochemical marker of pre-tumor changes.

So, the fungicide (benthialdicarb-isopropyl+folpet) has not induced multiplication or size increase of glutamyl transpeptidase nodules, which evidences about loss of the modifying effect of folpet on the benthialdicarb-isopropyl carcinogenicity in this preparation formula.

The obtained results may be used when deciding on administration of combined fungicides with active substances of one-purpose action.

Keywords: fungicides, γ -glutamyl transpeptidase, benthialdicarb-isopropyl, folpet, histochemical analysis, N-nitrosodimethylamine, N-Nitroso-N-Metylurea, N-nitrosobis (2-hydroxypropyl) amine, rats, promotor activity.

2. Methods

The study was held in Medved's Institute of Ecohygiene and Toxicology Center for preventive and regulatory toxicology (2015-2018 years), with 45 male wistar han rats, SPF, weighing 130±5.9 g. After being randomized, they were divided into 3 groups, where group 1 (negative control group) received water with polyoxyethylene isooctylphenol ether emulsifier (State Standard 8433-81) (Brand generic name «OP-10») (0.05 %); group 2 – received water suspension with the «OP-10» and preparation (benthialdicarb-isopropyl+folpet); and group 3 received water suspension with «OP-10» and triadimefon, which has hepatocarcinogenic properties and showed positive effect in the average term test [10]. The scheme of introduction of cancerogens and test substance is represented in **Table 1**.

The liver specimens for histochemical analysis were selected immediately after dissection of the rats, from which the 5 micrometer sections were prepared, using freezing microtome [11]. After leaving the sections in cold acetone, the histochemical reaction on determining the γ -glutamyl transpeptidase, a transformed hepatocyte marker, which forms nodules while proliferate, was conducted [10, 12, 13].

The appropriate for the standard unit (cm²) total area, quantity and size of the nodules make up main criteria for evaluating the studied preparation promotor effect. To evaluate the quantity and area of the nodules the authors used software «Densitan-2».

Table 1
Study scheme

Group	Weeks			
	0	0-2	2-4	4-24
1	Intra-abdominal injection, 100 mg/kg NDEA	Intra-abdominal injection twice a week, 20 mg/kg MNU	Drinking water with 0.01 % NBHPA solution	Intragastric administration of water + «OP-10»
2				Intragastric administration of preparation (benthialdicarb-isopropyl+folpet) + «OP-10»
3				Intragastric as ministration triadimefon+«OP-10»

Note: NDEA – N-nitrosodimethylamine; MNU – N-Nitroso-N-Metylurea; NBHPA N-nitrosobis (2-hydroxypropyl) amine; «OP-10» – polyoxyethylene isooctylphenol ether emulsifier (State Standard 8433-81) (0.05 %)

The statistical data were calculated using the Excel 2010, «Statistica 6.0, Stat Soft The product licensed to XP Russia 31415926535897» software, IBM SPSS Statistics Base v.22., descriptive statistics were used. We established the data distribution in the sample by Kolmogorov and Shapiro-Wilkinson methods. In the case of a normal distribution of signs expressed on an interval scale, the analysis was performed using parametric methods (Student's t-criterion) with normal distribution for independent samples, and in the absence of such – nonparametric criteria – Kruskal-Wallis (ANOVA) and Mann Whitney. The analysis of qualitative characteristics (tumor frequency and other pathological changes) was performed using the criterion χ^2 or a two-sided accurate Fisher criterion.

3. Results

The generalized histochemical study data are represented in **Table 2**.

Table 2
Average quantity and area of hyperplastic γ -glutamyl transpeptidase nodules in the rat liver

Statistic parameters	Area ² of the nodules mm ² /cm ²	Quantity ³ of the nodules/cm ²	Average area ² of the nodules, mm ²
Group 1. Negative control (n=10)			
M±m	0.76±0.67	93.34±68.81	0.007±0.003
Group 2. (benthiavalicarb-isopropyl+folpet) (n=10)			
M±m	0.812±0.64	94.68±72.06	0.008±0.002
% of the average in the control ¹	82	89	113
p	0.13 ³	0.34 ³	0.22 ⁴
Group 3. Positive Control, Triadimefon (n=10)			
M± m	1.42±0,88	134.53±71.90	0.01 02±0.002
% of the average in the control	187	144	151
p	0.13 ³	0.34 ³	0.01 ⁴

Notes: M – mean average; n – quantity of the studied animals; m – standard deviation; ¹ – % of the average characteristics of the «study/negative control»; ² – % of the median «study/negative control»; ³ – due to Kruskal-Wallis criterion; ⁴ – due to Mann-Whitney criterion

As the selection of the obtained results did not correspond to the normal distribution law, we have estimated medians of the parameters. When compared to the control, the statistically reliable increase of γ -glutamyl transpeptidase positive nodules' mean area was detected in 51 % of animals which received hepatocarcinogen triadimefon ($p \leq 0.01$).

The total specific area of the nodules per cm² and specific quantity of the nodules per cm² have not reliably changed, though they have increased compared to the control by 99 % and 67 %, respectively. The obtained data evidence about the appropriate model choice. Though, in the animals which received the drug (benthiavalicarb-isopropyl+folpet), the above-mentioned characteristics did not differ from negative control. The total specific area of the nodules per cm² and specific quantity of nodules per cm² have not reliably changed, as they decreased compared to the control by 18 % and 12 %, respectively. The study revealed reliable increase of γ -glutamyl transpeptidase positive nodules mean area by 12 %.

4. Discussion

The study was held using the model, offered by Ito N. [10], which is based on carcinogen initiation of hepatocytes that leads to their transformation in tumor cells. During proliferation of the carcinogen-transformed cells in the liver tissues at first there form premalignant loci, then- hyperplastic nodules. The transformed cells express the γ -glutamyl transpeptidase enzyme, which is a histochemical marker of premalignancy. The presence, quantity and size of hyperplastic γ -glutamyl transpeptidase positive nodules in liver cells are the main estimation criteria of the preparation promotor effect [11, 14].

The benthiavalicarb-isopropyl and folpet effect on the animal liver tissues wasn't associated with proliferation of the carcinogen-transformed cells and formation of hyperplastic nodules, expressing the γ -glutamyl transpeptidase, which is a histochemical pre-malignancy marker.

So, the fungicide (benthiavalicarb-isopropyl+folpet) didn't induce multiplication or size increase of glutamyl transpeptidase nodules, which evidences about loss of the modifying effect of folpet on the benthiavalicarb-isopropyl carcinogenicity in this preparation formula.

Literature data analysis [15] revealed that the single intragastric introduction of benthiavalicarb-isopropyl, dosed as 2000 mg/kg of body weight, didn't initiate hepatocarcinogenesis in rats of the «NDEA –hepatectomy» model. The rat liver cell proliferative activity increase was observed after administration of benthiavalicarb-isopropyl, dosed 10 mg/kg of body weight during 7 days (cumulative dose – 70 mg/kg) [15]. In the study, only 2 mg/kg of body weight (cumulative dose – 240 mg/kg) were introduced during chronic administration, so the nodules' increase in size was not statistically reliable.

Total increase of single malformations of various localization may be considered as a non-specific promotor effect of the combined fungicide.

The obtained results may be used when deciding on application of combined fungicides with active substances of one-purpose action.

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