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Toxic interaction of glyphosate herbicide and tau-fluvalinate insecticide on pheasant embryos

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Egy glifozát hatóanyagú herbicid és egy tau-fluvalinát hatóanyagú inszekticid együttes méreghatásának vizsgálata fácánembriókon

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ABSTRACT

In this study, we investigated the individual and combined toxic effects of a glyphosate-containing herbicide (Amega Up) and a tau-fluvalinate-containing insecticide (Mavrik 24 EW) on pheasant embryos, after immersion treatment of eggs. The emulsions of Amega Up with a concentration of 4.0% (360 g/l glyphosate) and Mavrik 24 EW (240 g/l tau-fluvalinate) with a concentration of 0.05% were used as test materials. The immersion treatment was performed on the first day of incubation, and the pheasant eggs were processed on day 21 of incubation. During the necropsy, the following parameters were recorded: body weight, the beak-hoof range, embryonic mortality and type and frequency of macroscopic developmental abnormalities. In the group treated with 4.0% Amega Up emulsion, the body weight values were lower and the beak-hoof range was shorter. The changes were significantly different as compared to the control. As a result of Mavrik 24 EW treatment, embryonic body weight and beak-hoof range decreased and embryonic mortality increased, however, the differences were not significant. During the study of the combined toxic effect with the test materials, a significant decrease in embryonic body weight and a statistically justified decrease also in the beak-hoof range were observed compared to the control group. The frequency of macroscopic developmental malformations increased to a statistically justified extent. The combined administration of the test materials increased the embryotoxicity compared to the individual toxicity tests which was presumably manifested as an additive toxic interaction.

Keywords: glyphosate, tau-fluvalinate, interaction, embryotoxicity, pheasant embryo

ABSZTRAKT

Vizsgálatunkban egy glifozát tartalmú herbicid (Amega Up) és egy tau-fluvalinát tartalmú inszekticid (Mavrik 24 EW) egyedi és együttes mérgező hatását vizsgáltuk fácánembriókban, bemerítéses kezelést alkalmazva a tojásokon. Vizsgálati anyagként az Amega Up 4,0%-os (360 g/l glifozát) és a Mavrik 24 EW (240 g/l tau-fluvalinát) 0,05%-os koncentrációjú emulzióját használtuk. A bemerítéses kezelést a keltetés első napján végeztük, a fácántojásokat a keltetés 21. napján dolgoztuk fel. A kórbonctani vizsgálat során a következő paramétereket rögzítettük: testtömeg-értékek, csőr-lábvég méretek, embrionális mortalitás és a makroszkópos fejlődési rendellenességek típusa és gyakorisága. Az Amega Up

4,0%-os emulziójával kezelt csoportban szignifikánsan kisebb testtömeg értékeket mértünk a kontrollcsoport értékeihez képest. Statisztikailag igazolható eltérést figyeltünk meg a kontrollhoz képest a csőr-lábvég méretekben is. A Mavrik 24 EW kezelés hatására csökkent az embrionális testtömeg és csőr-lábvég méret, valamint fokozódott embrionális mortalitás, azonban az eltérések nem voltak szignifikánsak. A kísérleti anyagokkal elvégzett együttes méreghatás vizsgálatai során szignifikáns testtömeg-csökkenést, és ugyancsak a csőr-lábvég méretek statisztikailag igazolható mértékű csökkenését tapasztaltuk a kontrollcsoporthoz képest. Az interakciós vizsgálatok során a kontrollhoz képest a malformációk előfordulásának gyakorisága statisztikailag igazolható mértékben emelkedett. A vizsgálati anyagok együttes alkalmazása az egyedi kezelésekhez képest fokozta az embriótoxicitást, amely additív jellegű toxikus interakcióban nyilvánult meg.

Kulcsszavak: glifozát, tau-fluvalinát, inteakció, embriótoxicitás, fácánembrió

INTRODUCTION

The human population has increased by about 4 billion people since 1950 (Bongaarts, 2009), and currently about 8 billion people are living on Earth (United Nations, 2022a). Based on estimates, the population of our planet will reach 9.7 billion people by 2050 (United Nations, 2022b). Nowadays, agriculture has to face a big challenge, the world's rapidly growing population has to be supplied with food while the proportion of cultivated land is decreasing. In 2013, the size of the cultivated area per capita in the world was 0.197 ha, which is expected to decrease to 0.18 ha by 2050 (Lal, 2016), since there are only limited areas suitable for crop production on our planet.

To increase the output of agricultural production, the amount of chemicals (plant protection products, fertilizers) used in agriculture has increased significantly in the last half-century (Pretty, 1995; Berny, 2007). Intensive plant production technologies require the use of chemical pesticides in order to achieve higher crop yields. Pesticides used in agricultural production do not only affect the target organisms (Aktar et al., 2009; Berny, 2007). Their excessive use and misuse can result in loss of biodiversity. Exposure to pesticides can impact to survival of the terrestrial and aquatic organisms (Mahmood et al., 2016). With the expansion of ecotoxicology, we have more and more knowledge that describes the adverse effects of chemicals on the wildlife surrounding humans (Sinkovitsné and Benkő, 1993).

The adverse effects of various chemical compounds on the environment must be monitored (Budai et al., 2003; Szabó et al. 2020), since their exposure usually occurs in a complex way, so we can count on the combined toxic effects of multiple chemical exposures (Varga et al., 1999; Juhász et al., 2005), among which we distinguish additive, synergistic, potentiated and antagonistic effects (James et al., 2000).

Glyphosate (N-(phosphonomethyl) glycine) is the most widely used herbicide with total effect (Dill et al. 2010). It is absorbed through the leaves of the weeds and reaches different parts of the plant by the sap flow. Effectively kills both seed-grown and perennial monocotyledonous and dicotyledonous weeds. It can be used on crop fields, horticultural crops, small gardens, urban areas, weed and shrub eradication along roads and railway embankments, and for drying stock (Kanissery et al., 2019) The herbicidal effect of glyphosate is based on the inhibition of 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS), which is a plant enzyme involved in the synthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan), and thus enzyme inhibition caused by glyphosate results in the death of plants (Herrmann and Weaver, 1999). EPSPS is not found in animals, so glyphosate as a selective target in plants is considered relatively non-toxic to animals, and adverse effects occur only at higher doses (Giesy et al., 2000; Helander et al., 2012; Battaglin et al., 2014; Ouyang et al., 2021).

Tau-fluvalinate is a pyrethroid-type contact and stomach poison insecticide that is uniquely gentle on bees and other beneficial insects. Pyrethroids prolong membrane depolarization by disrupting the function of voltage-gated sodium channels (Ray and Fry, 2006). As a result of its mode of action, tau-fluvalinate can cause excessive excitability, leading to changes in muscle function that can lead to paralysis or death of the target organisms. Pyrethroid insecticides are much more toxic to insects than to mammals or birds. Pyrethroids exert their toxic effects more effectively at low temperatures than at high temperatures, and this negative temperature dependence is the result of sodium channel sensitivity to pyrethroids being enhanced at lower temperatures. The rate of detoxification of pyrethroids in mammals or birds is much faster and more efficient than in insects due to the higher body temperature (Narahashi et al., 2007).

The study of the harmful effects of environmental chemicals on wild birds is an extensively examined topic in ecotoxicology studies, but they focus primarily on the post-hatching life stage (Heinz et al., 1999; Spahn and Sherry, 1999). Exposure may occur earlier, during embryonic development. Eggs from wild birds can come into direct contact with various environmental chemicals (Kertész et al., 2006). Spring chemical crop protection works often coincide with the nesting period of wild birds. During the nesting period, environmental chemicals on the breast feathers, feet, or nesting materials of wild birds may be transferred to their eggs, causing embryonic mortality, growth impairment and/or teratogenicity (Kertész and Fáncsi, 2003).

Teratological testing on bird embryos is inexpensive, and fast, and the increased sensitivity of avian embryos allows the investigation of chemical exposure on fetal development. Another advantage of the method is that the embryonic development of birds is close to the development of mammals in many morphological and functional aspects (Hill and Hoffman, 1984). The main disadvantage of this type of examination is the absence of a maternal-fetal relationship and the fact that the avian embryo may react too sensitively to certain agents in some cases (Wilson, 1978). In avian teratological tests, the injection or immersion treatment method is most often used. The advantage of the injection treatment method is that the test substance can be injected into any part of the egg in a precisely measured dose. The immersion treatment method is justified for environmental chemicals (e.g. pesticides) as it is better model for exposure in nature (Juhász et al., 2006).

The results of teratological studies on avian embryos are useful for environmental risk assessment of chemicals, which is essential for the protection of wild birds and their offspring (Várnagy et al., 1996).

Our study aimed to determine the individual and combined toxicity of a widely used, glyphosate-based herbicide, Amega Up, and a pyrethroid-based tau-fluvalinate insecticide marketed as Mavrick 24 EW in the development of pheasant embryos. Since the test methods used in ecotoxicology are primarily aimed at the investigation of individual toxic effects, the data on the interactions between pesticides can be considered highly important and topical, especially in relation to the bird organism.

MATERIALS AND METHODS

To model the environmental load caused by plant protection products, the concentration of the pesticides used in the experiment corresponds to that usually applied during chemical plant protection. Both during individual and combined treatment, the 4% spray concentration of Amega Up (Nufarm Hungária Ltd., Hungary) with 360 g/l glyphosate active ingredient, and 0.05% emulsions of Mavrik 24 EW (240 g/l, Nufarm Hungária Ltd., Hungary) containing tau-fluvalinate were used. The pheasant (*Phasianus colchicus*) eggs with good fertility used in our experiments originated from the pheasant farm of the Hubertus Hunting Association located in Abádszalók (Abádszalók, Hungary).

After delivery, the pheasant eggs were rested for 24 hours. In the case of pheasants, the incubation period is 24 days. The first 20 days are the set stage, while the last 4 days are the hatch stage (Nagy, 1994). During the entire incubation period, we ensured the appropriate temperature and humidity and ensured that the eggs were rotated. The heat requirement for the set stage is 37.8 °C, with a relative humidity of 48.0-51.0%. The heat requirement for the hatch stage is 37.5 °C, with a relative humidity of 65-80% (Nagy, 1994).

The pheasant eggs were randomly divided into four homologous groups (60 eggs/group) based on their size and weight. Pheasant eggs were incubated in a RAGUS® table incubator (Wien, Austria).

The study and the treatments were carried out accordingly with the methods described by Várnagy et al. (2000). All applicable international, national and/or institutional guidelines for the care and use of animals were followed. The experimental protocol of the study was approved by the local Committee of Animal Welfare at the Hungarian University of Agriculture and Life Sciences, Georgikon Campus (permission No.: MÁB-3/2023).

The individual and combined treatment with test materials was carried out immediately before the start of incubation. The pheasant eggs were immersed into the emulsion of the test materials (Amega Up 4.0% emulsion, Mavrik 24 EW 0.05% emulsion) at 37 °C for a period of 30 minutes. After the immersion treatment, the incubation was started.

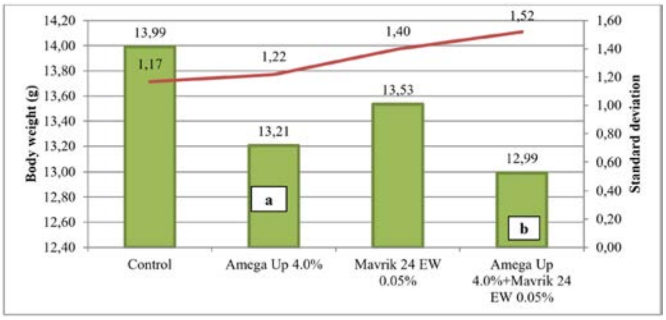
The pheasant eggs were opened on day 21 of incubation, 3 days before the expected hatching. During the necropsy, the following parameters were recorded: the body weight, the beak-hoof range, the type of macroscopic developmental abnormalities and their frequency of occurrence. Furthermore, the number of dead embryos and the time of embryonic death were also determined. Dawson's procedure was used to stain the skeleton of the pheasant embryos to examine developmental abnormalities (Dawson, 1926). The stained preparations were evaluated with the naked eye and a stereomicroscope.

The distribution of the body weight data and the beak-hoof range length of the live embryos were checked graphically with a Comparison-Quantile Plot, while the statistical evaluation was performed with a one-way ANOVA. Pairwise comparisons were made with the TUKEY HSD test. Fisher's exact test was used for the biometric processing of developmental abnormalities and embryo mortality data.

RESULTS

Embryonic body weight

The means of the body weight of the pheasant embryos measured in the study are presented in Figure 1. In the control group, the average body weight of the pheasant embryos was 13.99 ± 1.17 g. The body weight of the pheasant embryo (13.21 ± 1.22 g) after single administration of glyphosate-based Amega Up at a concentration of 4% was significantly different ($P < 0.05$) from the values measured in the control group. In the group individually treated with the 0.05% emulsion of Mavrik 24 EW containing tau-fluvalinate, the body weight of the pheasant embryos was 13.53 ± 1.40 g, which was insignificant compared to the control group. Combined administration of glyphosate-based Amega Up at a concentration of 4% and the tau-fluvalinate-based Mavrik 24 EW at a concentration of 0.05% resulted in the lowest body weight values (12.99 ± 1.52 g), which was significantly ($P < 0.01$) lower compared to the body weight data measured in the control group.



^{a} Significant decrease as compared to the control data ($P < 0.05$)

^{b} Significant decrease as compared to the control data ($P < 0.01$)

Figure 1. Body weight (average ± SD; g) of the embryos in the avian teratological test of single and combined administration of Amega Up and Mavrik 24 EW

Embryonic mortality

The results of the embryonic mortality are presented in Table 1. In the control group, 3 dead pheasant embryos were registered during the processing, the proportion of which compared to fertile eggs was 5.66%.

Table 1. Mortality and developmental anomalies of embryos in the avian teratological test of single and combined administration of Amega Up and Mavrik 24 EW

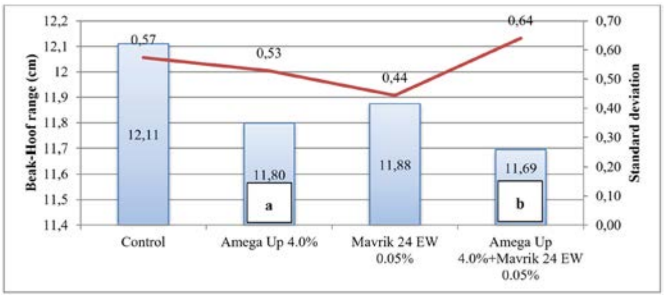
Treatment	No. of embryos showing anomaly/ No. of live embryos	No. of death/ Total fertile eggs	Rate of embryos showing anomaly (%)	Rate of dead embryos (%)	Rate of live embryos (%)
Control	0/50	3/53	0	5.66	94.34
Amega Up 4.0%	3/47	5/52	6.38	9.62	90.38
Mavrik 24 EW 0.05%	4/48	5/53	8.33	9.43	90.57
Amega Up 4.0% + Mavrik 24 EW 0.05%	6/44[a]	9/53	13.64	16.98	83.02

[a] Significant decrease compared to the control data ($P < 0.001$)

The application of glyphosate-based Amega Up herbicide at a concentration of 4% caused 5 dead embryos, the proportion of which was 9.62% compared to all fertile eggs. As a result of a single treatment with the insecticide Mavrik 24 EW at a concentration of 0.05%, 5 dead embryos (9.43%) were noted. Nine dead embryos (16.98%) were recorded during the processing after the combined application of glyphosate-based Amega Up at a concentration of 4% and the tau-fluvalinate-based Mavrik 24 EW at a concentration of 0.05%, which was three times higher than that of the values registered in the control group. Although the number of dead embryos increased in all treated groups, the differences were not significant in any case compared to the control group.

The beak-hoof range

In the pheasant embryos of the control group, the mean length from the tip of the beak to the tip of the middle toe was 12.11 ± 0.57 cm (Figure 2). Due to the single administration of 4% Amega Up the beak-hoof range (11.80 ± 0.53 cm) significantly decreased ($P < 0.05$) compared to the control (Figure 2). The mean value of the beak-hoof range of the pheasant embryos (11.88 ± 0.44 cm) in the group that had been administered with Mavrik 24 EW at a concentration of 0.05% showed a statistically unjustified difference compared to the control group. The combined administration of Amega Up at a concentration of 4% and Mavrik 24 EW at a concentration of 0.05% resulted in a significant decrease ($P < 0.01$) of the average value of the beak-hoof range length (11.69 ± 0.64 cm) as compared to the control.



[a] Significant decrease as compared to the control data ($P < 0.05$)

[b] Significant decrease as compared to the control data ($P < 0.01$)

Figure 2. Beak-hoof range (average ± SD; g) of the embryos in the avian teratological test of single and combined administration of Amega Up and Mavrik 24 EW

Developmental anomalies

Developmental anomalies were not recorded in the control group (Table 1). Three embryos (6.38%) were observed with developmental anomalies in the group with a single treatment of 4% Amega Up, which was manifested in leg deformation in all cases. Leg deformation was observed on 4 embryos (8.33%) in the group treated with a single treatment of 0.05% Mavrik 24 EW. As a result of the combined treatment of Amega Up at a concentration of 4% and Mavrik 24 EW at a concentration of 0.05%, the number of pheasant embryos showing macroscopic developmental abnormalities was increased to 6 (13.64%), which was a statistically verifiable difference (*P* < 0.001) compared to the control group. Leg deformation was observed in all cases of individuals showing deformity.

DISCUSSION

Amega Up applied at a concentration of 4% proved to be embryotoxic, its harmful effects on pheasant embryos were manifested in a decrease in body weight compared to the control group and a significant decrease in the beak-hoof range. The number of embryos showing development anomalies and the rate of embryonic mortality in the group treated with the herbicide alone increased compared to the control. However, the differences were not significant. As the rate of developmental abnormalities was sporadic, no teratogenic effect was justified.

Similar results were reported by Pintér et al. (2018) in their embryotoxicity study with Amega (glyphosate 360 g/l) herbicide. Due to the single treatment with Amega at a concentration of 2%, the body weight values of the pheasant embryos were significantly lower compared to the control. There was a small increase in embryonic mortality and the number of embryos showing developmental malformations.

Adverse effects of the glyphosate-based herbicide Roundup which contained polyethoxylated tallow amine on the reproductive system of ducks (*Anas platyrhynchos*) were examined by Oliveira et al. (2007). Due to the Roundup treatment, morphological changes in the structure of the testis and epididymis, decreased androgen receptor expression in the testis, and reduction in the testosterone and estradiol levels were detected.

Paganelli et al. (2010) studied the effect of glyphosate-based herbicides on the embryonic development of chicken embryos, using the injection treatment method. During their studies, they observed that 20 µl of 1/3500 and 1/4500 dilution emulsion of the herbicide increased the occurrence of developmental anomalies.

Ruuskanen et al. (2020) used Japanese quails (*Coturnix coturnix japonica*) as wild bird model to study the adverse developmental effects of Roundup Flex (glyphosate 480 g/l) on ecologically relevant traits, feather development, and body weight gain. The food for the quails was prepared by adding Roundup Flex. The concentration of glyphosate in the Roundup Flex treated food was 160 mg/kg food. Consumption of food prepared with the test material resulted in a delay in plumage development and residues of glyphosate-based herbicides were present in eggs, liver, and breast muscle. However, only slight adverse effects on embryo development and physiology can be induced by a 200 mg/kg dose of Round Flex mixed into the feed (Ruuskanen et al., 2022).

A retrospective analysis of available literature on glyphosate stated that glyphosate exposure has no consistent effect on reproduction, fertility, or development of the offspring. The results of toxicity studies performed with glyphosate-based products indicate that adverse effects are due to the surfactants present in the products and are not a direct consequence of glyphosate treatment (Williams et al., 2000, 2012).

However, many toxicology studies have been conducted on the toxic effects of glyphosate and glyphosate-base herbicides, which show that glyphosate and glyphosate-based formulation have cardiotoxic (Gress et al., 2015), gastrointestinal (Rueda-Ruzafa et al., 2019), hepatotoxic (Mesnage et al., 2017) and nephrotoxic (Gao et al., 2019) effects.

Numerous *in vitro* studies were conducted with glyphosate and glyphosate-containing herbicides, which showed cytotoxic, neurotoxic, carcinogenic, genotoxic, teratogenic, endocrine disrupting effects, but these toxic effects were not confirmed in *in vivo* studies on mammals. Based on the results of *in vivo* experiments, several international institutions, including the EFSA, do not consider glyphosate to be a neurotoxic, carcinogenic, genotoxic, teratogenic, or endocrine-disrupting substance (Soares et al., 2021).

Based on the experimental design used in laboratory tests, it is often not possible to distinguish the possible effects of the co-formulants themselves, or whether they have changed the effect of the active ingredient, glyphosate.

The main components of glyphosate-based herbicides are glyphosate salts as active ingredients, and surfactants and water as co-formulants (Martens et al., 2019). In the case of some pesticides, the toxicity of surfactants can be greater than that of the active ingredients (Lehel et al., 2021). Previously, the most common co-formulants of glyphosate-based herbicides were polyethoxylated tallow amine (POEA) surfactants belonging to the first generation of polyethoxylated amines, which proved more toxic than glyphosate active ingredient (Mesnage et al., 2019). Based on the recommendation of the European Commission, EU member states banned the use of POEA-type surfactants in glyphosate-based herbicides (Mesnage and Antoniou, 2018). In the glyphosate-based Amega Up herbicide used in our study, instead of POEA, a toxicologically irrelevant polyethoxylated derivative is found as a co-formulant.

The majority of toxicological experiments investigating the effect of glyphosate and glyphosate-based herbicides were carried out in laboratory conditions, in which the applied treatments differ, to a greater or lesser extent, from the exposures prevailing in the natural environment, both in terms of doses and treatment methods.

In the group individually treated with the 0.05% emulsion of Mavrik 24 EW, no significant body weight loss was detected compared to the control group. The 0.05% emulsion of the insecticide proved to be slightly embryotoxic, but not considered teratogenic during the treatment. The embryotoxic effect was manifested in a decrease of the beak-hoof range, in increasing embryonic mortality, and in a statistically unjustified increase in the number of malformations compared to the control.

Anwar (2003) investigated the toxic effect of pyrethroid-type cypermethrin on chicken embryos. On day 0 of incubation, 0.05 ml of the test substance was injected into the eggs at a concentration of 50, 100, 200 and 400 mg/kg. On day 7 of processing, as a result of cypermethrin treatment at concentrations of 100, 200 and 400 ppm, the following teratogenic changes appeared as a decrease of crown-rump length, size of brain and eyeballs, and incomplete development was observed on the eyes, beak and wing buds.

Uggini et al. (2012) studied the embryotoxic and teratogenic effect of the insecticide Anaconda 505™ containing chlorpyrifos (50%) and cypermethrin (5%) as active ingredients on chicken embryos. The injection treatments were performed on day 0 of hatching, and the examination of developmental abnormalities was performed on live hatchlings and dead embryos. The results of their experiment revealed that the Anaconda 505™ insecticide produced characteristic teratogenic effects, manifested in morphological and skeletal malformations.

Verma et al. (2021) studied the joint toxic effect of chlorpyrifos and cypermethrin combination on the development of the heart in chick embryos. Their study results suggest that maternal exposure to chlorpyrifos and cypermethrin combination increases the risk of congenital heart defects in offspring.

David (1981) studied the repellent properties of the active ingredient decamethrin, a decamethrin-based insecticide and co-formulant in Japanese quail (*Coturnix coturnix japonica*). The test substances were dispersed on the surface of the feed at a dose of 100 ppm. The experiment results showed that the feed treated with deltamethrin-based insecticide had a repellent effect on the quail, and the smell emitted by the pesticide was repellent to the quail.

Kaneko et al. (1992) investigated the embryotoxic effect of empenthrin, which belongs to the group of pyrethroid insecticides, in mammalian test organisms. Rats were treated orally with doses of 50, 150 and 500 mg/kg, while rabbits with doses of 100, 300 and 1000 mg/kg during the period of organogenesis. Maternal toxicity occurred in rats at 500 mg/kg and in rabbits at 300 mg/kg or higher. Fetal developmental anomalies were not observed in either rats or rabbits. Empenthrin is not teratogenic in the tested mammals.

At the same time, pyrethroids are highly toxic to aquatic invertebrates and vertebrates, which results in low body temperature, differences in metabolism, and more significant damage to mitochondrial energy-producing processes (Chrustek et al., 2018).

Cypermethrin and permethrin produced dose-dependent teratogenic effects on European sea bass (*Dicentrarchus labrax* L, 1758) embryo-larval stages including developmental abnormalities such as pericardial oedemata, yolk oedemata, scoliosis, kyphosis, lordosis, lack of eyes, body atrophy and cranial deformation (El Ayari et al. (2022).

Pyrethroids are much more toxic to insects than to terrestrial vertebrates or humans because the sodium channels of insects are more sensitive, their body size is significantly smaller and their body temperature is much lower. Among terrestrial vertebrates, birds have the highest body temperature, this fact is significant in the selective toxicity of pyrethroid insecticides since higher body temperature is significantly positively correlated with the intensity of metabolic processes and negatively correlated with the sensitivity of voltage-gated sodium channels to pyrethroids (Rehman et al., 2014; Ranatunga et al., 2023).

The simultaneous presence of different plant pests may make it necessary to apply herbicides and insecticides together in the form of a tank mixture. The adverse effect resulting from the interaction of different pesticides on avian embryos is a quite rarely studied topic.

In our experiments, the results of combined administration of Amega Up at a concentration of 4% and Mavrik 24 EW at a concentration of 0.05% showed that embryonic mortality and the incidence rate of malformations were increased, at the same time the body weight and the beak-hoof range were decreased significantly compared to the control groups.

Based on the available literature, it can be concluded that the joint toxic effect of many pesticide combinations manifests at least in an additive form, but in the case of certain combinations, the joint toxic effect can significantly exceed the summation of the individual toxic effects.

CONCLUSIONS

Based on the results of the teratological tests performed on pheasant embryos with the herbicide Amega Up and the insecticide Mavrik 24 EW, the combined toxic effect of both pesticides increased the embryotoxicity, which represented in significant decrease in body weight of the embryos, decreased the beak-hoof range, and increase of embryonic mortality under the conditions used in our experiment. The combined toxic effect of glyphosate-based Amega Up and tau-fluvalinate-based Mavrik 24 EW induced an additive effect compared to the individual toxicity of the test formulations.

Generally, the individual chemical agents are mainly used separately in the ecotoxicological examination of various chemical substances, however, the chemical load can usually occur in a complex way, so it is possible to count on the combined toxic effect of the chemical substances present at the same time, as a result of which the chemical substances interact with each other. The results of chemical interaction toxicity studies show that joint exposure to different chemicals (e.g. pesticides) can modify the toxicity of the chemical components, particularly the increase of it resulting in synergism or addition, which multiplies the risk of their use.

However, it should not be overlooked that the combined adverse effects are influenced by many factors: exposure parameters (dose, duration, frequency) and biological characteristics of the living organism, such as species, sex, age, health status, etc. These factors must be taken into account when predicting the toxic effects of natural exposures on environmental living organisms based on the results of ecotoxicological experiments.

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