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# TOXICITY TEST OF INDIVIDUAL AND COMBINED TOXIC EFFECTS OF GLYPHOSATE HERBICIDE AND HEAVY METALS ON CHICKEN EMBRYOS

Rita SZABÓ[1]*, Dalma CSONKA[1], László MAJOR[1], József LEHEL[2], Péter BUDAI[1]

[1]University of Pannonia, Georgikon Faculty, Institute of Plant Protection, Hungary
[2]University of Veterinary Medicine, Department of Food Hygiene, Hungary
*Corresponding author: szabo-r@georgikon.hu

## ABSTRACT

The aim of this study was to determine the individual and combined toxicity of Glialka Star herbicide (glyphosate 360 g/l) and heavy metals (copper and cadmium) on the development of chicken embryos. On the first day of incubation, chicken eggs were injected by 0.1 ml of the test materials. The applied concentration of copper and cadmium sulphate was 0.01% and that of herbicide Glialka Star was 2%. The chicken embryos were examined on day 19 by the followings: rate of embryo mortality, body mass, type of developmental anomalies by macroscopic examination. The body weight was evaluated statistically by one-way ANOVA combined with Dunnett post-test, the embryo mortality and the developmental anomalies were analysed by Fisher test. Our teratogenicity study revealed that the combined administration of heavy metals (copper, cadmium) and glyphosate (K-salt) containing herbicide formulation caused significant reduction in the body weight of embryos and a significant increase in the rate of embryonic mortality and the incidence of developmental anomalies. The joint toxic effect of heavy metals and Glialka Star is an additive effect compared to the individual toxicity of the test materials.

**Keywords:** *glyphosate, heavy metals, interaction, embryotoxicity, chicken embryo.*

## INTRODUCTION

Because the land used for agricultural production is the food source, shelter and breeding place of our wild birds, the pesticides sprayed during the plant protection activities might have influence not only on mature birds but also on the embryos within the eggs. In the environment which is contaminated by pesticides, these materials change the chemical environment of animals and make poisonings possible. The harmful effects of pesticides can appear in acute damage, or destruction of living creatures at a lower or higher level. The destruction of the mature animals can cause the death of the offspring which remain without food and care, even if they are not poisoned. Those who survive the acute poisoning with a decreased resistance can become the victims of different environmental pollution (Várnagy and Budai, 1995).

Recently, the examination of the combination of heavy metals and other chemicals has gained significant ground in both avian (Fejes *et al.*, 2001; Kertész, 2001) and mammalian (Institóris *et al.*, 2001; Pecze *et al.*, 2001) toxicology research studies.

Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environmentally friendly chemical plant protection techniques (Várnagy *et al.*, 1996).

The objective of this study was to determine the individual and combined embryotoxic effects of heavy metals (copper, cadmium) modelling the heavy metal load of the environment and an optionally selected pesticide widely applied in the practice (Glialka Star). As the ecotoxicological testing methods used in the practice are mainly limited to study the toxic effect of compounds used alone, data on interactions between pesticides can be regarded as gap-filling information especially in relation to the avian organism.

## MATERIALS AND METHODS

For modelling the environmental copper and cadmium load, 0.01% copper sulphate solution (Reanal-Ker Ltd., Hungary) and 0.01% cadmium sulphate solution (Reanal-Ker Ltd., Hungary) was used in individual and joint treatments.

At present, copper is used primarily for wire manufacturing, as a chemical catalyst and for the production of alloys. It is applied as a nutrient in plant cultivation and as a bactericidal, fungicidal and algicidal agent in chemical plant protection. In veterinary medicine copper is used as a feed additive, growth promoter and disease-preventing substance. Cadmium is used in the industry for the production of alloys and for corrosion protection of iron objects. It is also used in the manufacture of batteries, rubber and paint, as well as to stabilize plastics. However, it is also not unknown in the field of chemical plant protection that cadmium-containing compounds have previously been used as fungicides on golf courses (Adriano, 1986).

The herbicide Glialka Star (360 g/l glyphosate [K-salt], Monsanto Hungária Ltd., Budapest, Hungary) was used in individual and joint treatments in typical field application rate (2%). It is a phosphorous-containing pesticide with 360 g/l glyphosate K-salt as active ingredient and assigned to marketing category III. It is used widely on arable land as well as in horticulture and viticulture, for drying and killing annual and perennial single and dicotyledonous plants. The product is not toxic to bees and to fish (NFCSO, 2011). The studies were conducted with purebred fertile Farm hen's eggs derived from the stock farm of Goldavis Ltd. (Sármellék, Hungary). The eggs were incubated in a Ragus type hatcher (Vienna, Austria). During the incubation, the appropriate temperature (37–38°C), air humidity (65–75%) and the daily rotation of eggs were provided (Bogenfürst, 2004). The treatment of eggs (n=45/group) was performed on the day of initiation of hatching. In the individual treatments, solutions and emulsion made from test chemicals in 0.1–0.1 ml end volume were used while in the joint treatments, 0.2 ml of the chemical agents were injected into the air chambers of eggs in each combination (Clegg, 1964; Lutz, 1974). For the preparation of solutions and emulsion as well as in the control treatments, distilled water was used. The incubation was started immediately after the treatments. The processing was conducted two days before the expected hatching on the 19$^{th}$ day of incubation. Within the pathological studies, the body weight of embryos, the number of dead embryos and the macroscopic malformations were determined and recorded. In the case of the body weight data of live embryos, statistical comparisons among the groups were made with one-way analysis of variances (ANOVA). Because the results showed significant differences, Dunnett tests were also performed. In the case of the biometric processing of the embryonic mortality and malformations, exact test according to Fisher was used.

## RESULTS AND DISCUSSION

The embryonic mortality in the control group treated with distilled water was 4.65% (Table 1). The rate of embryonic mortality could be considered sporadic which made it possible to use that group as a frame of reference. There was no any malformation in this group (Table 2). On the effect of the injected distilled water in the control group, the average body weight of the embryos was 23.24±1.85 g (Table 3).

On the effect of the 0.01% copper sulphate solution the embryonic mortality significantly ($p<0.05$) increased to 20.45% in comparison to the control group (Table 1). Malformations occurred two times in that group (5.71%). Types of developmental abnormalities were the followings: open chest cavity, retarded development, and malformation of feet (Table 2). Injection of copper sulphate decreased the body weight of embryos (22.56±2.06 g) in comparison to the control group (23.24±1.85 g), but the difference could not be proved statistically (Table 3).

When the cadmium sulphate was used individually, almost one half of the treated animals died (47.73%; $p<0.001$) that was statistically significant compared to the control group (Table 1). There was not any embryo showing developmental anomalies in the group (Table 2). The individual administration of cadmium sulphate in 0.01% concentration on the day 0 of the incubation caused a significant ($p<0.05$) reduction in the body weight of embryos (21.86±2.25 g) compared to the control data (Table 3).

The rate of embryonic mortality significantly ($p<0.01$) increased (30.95%) in the group treated with herbicide (Glialka Star) individually (Table 1). When the glyphosate-containing herbicide was used the incidence of developmental anomalies was sporadic (3.44%) (Table 2). The developmental anomalies appeared as malformation of feet, open body cavity and retarded growth. After the single administration of the herbicide formulation in 2% concentration on the day 0 of the incubation, significant decrease ($p<0.01$) was established in the embryonic body weight (21.84±1.70 g) in comparison with the values of the control group (23.24±1.85 g), (Table 3).

As a result of the combined application of copper sulphate and the herbicide, the rate of embryonic mortality reached 53.48% that was statistically significant compared to the control group ($p<0.001$) (Table 1). Three of the living embryos (3/20) as a result of combined administration showed developmental anomaly. The incidence of developmental anomalies was significantly ($p<0.05$) higher than the control. Looking at the types of development disorders the most frequent problem was retarded development, malformation of feet, lack of right eye, bulging of left eye, the shortening of the beak mandible, and open chest cavity (Table 2). The combined administration of copper sulphate and glyphosate containing herbicide formulation resulted in a significant decrease in the average body weight (21.05±1.73 g) of embryos compared to both the control group ($p<0.001$; 23.24±1.85 g) and the group treated with either copper sulphate ($p<0.01$; 22.56±2.06 g) alone (Table 3).

In comparison with the values of the control group, the rate of dead embryos increased significantly ($p<0.001$) in the group treated with cadmium sulphate and glyphosate-containing herbicide product (Glialka Star). The rate of embryonic mortality was 83.33% (Table 1). In the group the occurrence of development disorders (42.85%) was at a significant higher level ($p<0.01$) than in the case of individual treatments (cadmium sulphate: $p<0.01$; Glialka Star: $p<0.05$). The developmental anomalies could be identified as hernia of brain, retarded development, malformation of feet, open body cavity, bulging of eye (Table 2). The combined treatment with cadmium sulphate and Glialka Star induced significant decrease of the embryonic body mass (19.13±1.35 g; $p<0.001$) compared with the control and individually treated groups ($p<0.01$) (Table 3). So, it can be established that the combined treatment resulted in an increased embryotoxic effect in comparison with the individual embryo damaging effect of the used compounds separately.

In former teratogenicity tests (Fejes, 2005) performed on chicken embryos using a series of concentrations of copper and cadmium sulphate solution (1.0%, 0.1%, 0.01%, 0.001%) the rate of embryonic mortality and developmental anomalies proved to be significantly ($p<0.05$) increased in comparison to the control groups.

In teratogenicity studies of various copper salts conducted on pregnant hamsters it was found that the high doses of copper sulphate had increased the rate of embryonic mortality, malformations in uteri and foetal deformities. Malformation of the heart appeared to be a of the tested copper compounds (Ferm and Hanlon, 1974).

Similarly, to previous results (Juhász *et al.*, 2006; Szabó *et al.*, 2011), according to the pathological studies it was established that individual treatments with cadmium sulphate significantly ($p<0.05$) increased the embryonic mortality.

Based on the results of our teratogenicity studies on single administration of injected copper sulphate, it can be established that at 0.01 concentration level has a slight embryotoxic effect. The teratogenic effect was not justified. On the base of our teratogenicity studies on single administration of injected glyphosate containing herbicide formulation (Glialka Star) caused a significant reduction in the body weight of embryos and markedly increased the rate of embryonic mortality. The herbicide Fozát 480 (glyphosate) in individual and combined administrations significantly increased the embryonic mortality (Szabó *et al.*, 2017).

Table 1. The number and rate of dead embryos on day 19 of incubation

| Treated groups | Number of dead embryos/ number of fertile eggs (pcs) | Rate of dead embryos (%) |
|---|---|---|
| Control | 2/43 | 4.65 |
| Copper sulphate | 9/44[a1] | 20.45 |
| Cadmium sulphate | 21/44[a3] | 47.73 |
| Glialka Star | 13/42[a2] | 30.95 |
| Copper sulphate + Glialka Star | 23/43[a3, b] | 53.48 |
| Cadmium sulphate + Glialka Star | 35/42[a3, c, d] | 83.33 |

[a]Significant difference as compared to the control group ([a1]$p<0.05$; [a2]$p<0.01$; [a3]$p<0.001$).
[b]Significant difference as compared to the group treated with copper sulphate alone ([b]$p<0.05$).
[c]Significant difference as compared to the group treated with cadmium sulphate alone ([c]$p<0.05$).
[d]Significant difference as compared to the group treated with Glialka Star alone ([d]$p<0.01$).

Table 2. Number and rate of malformed embryos on day 19 of incubation

| Treated groups | Number of malformed embryos/ number of alive embryos (pcs) | Rate of malformed embryos (%) |
|---|---|---|
| Control | 0/41 | 0.00 |
| Copper sulphate | 2/35 | 5.71 |
| Cadmium sulphate | 0/23 | 0.00 |
| Glialka Star | 1/29 | 3.44 |
| Copper sulphate + Glialka Star | 3/20[a1] | 15.00 |
| Cadmium sulphate + Glialka Star | 3/7[a2, b, c] | 42.85 |

[a]Significant difference as compared to the control group ([a1]$p<0.05$; [a2]$p<0.01$).
[b]Significant difference as compared to the group treated with cadmium sulphate alone ([b]$p<0.01$).
[c]Significant difference as compared to the group treated with Glialka Star alone ([c]$p<0.05$).

Table 3. Embryonic body weights (g) on day 19 of incubation

| | Control | Copper sulphate | Cadmium sulphate | Glialka Star | Copper sulphate + Glialka Star | Cadmium sulphate + Glialka Star |
|---|---|---|---|---|---|---|
| Number of embryos (n) | 41 | 35 | 23 | 29 | 20 | 7 |
| Average (g) | 23.24 ±1.85 | 22.56 ±2.06 | 21.86 $\pm 2.25^{a1}$ | 21.84 $\pm 1.70^{a2}$ | 21.05 $\pm 1.73^{a3, b}$ | 19.23 $\pm 1.35^{a3, c, d}$ |

$^{a}$Significant difference as compared to the control group ($^{a1}p<0.05$; $^{a2}p<0.01$; $^{a3}p<0.001$).
$^{b}$Significant difference as compared to the group treated with copper sulphate alone ($^{b}p<0.01$).
$^{c}$Significant difference as compared to the group treated with cadmium sulphate alone ($^{c}p<0.01$).
$^{d}$Significant difference as compared to the group treated with Glialka Star alone ($^{d}p<0.01$).

## CONCLUSION

Our teratogenicity study revealed that the combined administration of injected copper and cadmium sulphate as well as glyphosate-containing herbicide product (Glialka Star) caused a significant reduction in the body weight of the embryos and markedly increased the rate of embryonic mortality. The combined toxic effect of copper sulphate and glyphosate, and cadmium sulphate and glyphosate induced additive effect compared to the individual toxicity of the test materials. The test conducted on day 19 of incubation showed a significant increase in embryonic mortality when heavy metals and herbicide were applied individually. The occurrence of developmental abnormities in the groups receiving individual treatments remained at a relatively low level (3.44-5.71%). However, due to the combined treatment the rate of malformed embryos was highly increased (15.00-42.85%) resulted in synergistic effect. The rate of mortality due to the combined administration was definitely increased over 50% compared to the individual toxicity of heavy metals and the herbicide. In the groups treated with both cadmium sulphate and Glialka Star the body weight of embryos showed a significant decrease compared to both the control and individually treated groups.
Beside the injection treatment method applied during the studies it would be advisable to perform complete examinations with spraying (or bathing, immersing) treatments that can be served as a model of expositional circumstances during the plant protection practice and compare the results achieved from different treatment technologies.

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