Original scientific paper
10.7251/AGRENG1703037S
UDC: 632.954:[591.3:636.52./.58]
546.48:[591.3:636.52./.58]

STUDIES ON JOINT TOXIC EFFECTS OF A GLYPHOSATE HERBICIDE (FOZÁT 480) AND A HEAVY METAL (CADMIUM) ON CHICKEN EMBRYOS

Rita SZABÓ[1]*, Géza SZEMERÉDY[1], Éva KORMOS[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 the study was to determine the individual and combined toxic effects of the herbicide Fozát 480 (glyphosate [isopropylamine salt] 480g/l) and cadmium sulphate ($CdSO_4$) on the development of chicken embryos. On the first day of incubation, chicken eggs were injected with 0.1 ml of cadmium sulphate solution (0.1%) and/or with 0.1 ml of Fozát 480 (2.0%). The chicken embryos were examined on the 19th day by measuring the rate of embryo mortality and body weight and by identification of different types of developmental anomalies and macroscopic malformations. The body weight data were statistically evaluated by one-way ANOVA and Dunnett tests, while the embryonic mortality and the developmental anomalies were analysed by Fisher test. Our teratogenicity study revealed, that the combined administration of cadmium sulphate and glyphosate (isopropylamine salt) containing herbicide formulation caused a significant reduction in the body weight of embryos and increased the rate of embryonic mortality. The joint toxic effect of cadmium sulphate and Fozát 480 is an additive effect compared to the individual toxicity of the test materials.

Keywords: *glyphosate (isopropylamine salt), cadmium sulphate, joint toxic effect, ecotoxicology, chicken embryo.*

INTRODUCTION

Preservation of natural values and protection of the environment are integral parts of social life and economy. Environmental pollution, exploitation of natural resources and imbalances in the dynamic equilibrium of living systems may lead to the constricting of living spaces and expose our health and safety to danger in the short run. Nowadays, one of the most important basic principles is to produce high quality safe food that indispensably requires clean environment. The European Union pays particular attention to the environmental protection and attaches great importance to environmentally sound management. In addition to the production of healthy foods, agricultural production have to minimize the environmental load while promoting the energy-efficient use of non-renewable raw materials. Unfortunately, there are still large quantities of chemicals in our environment of which biological activity is not fully known.

Because of the rapid industrial and agricultural development, humans and the surrounding environment suffers from a significant chemical load within which pesticides play an important role. Although pesticide use helps fighting off harmful organisms providing higher yield safety, all chemicals are potential poisons and present serious risk. The fauna of the cultivated areas and humans using the pesticides are primarily exposed to chemical loads. Agricultural lands provide food source, shelter and nesting place for our wild fowls. Pesticides sprayed during plant protection have not only impacts on adult fowls but also on the embryos developing within the eggs (Várnagy and Budai, 1995). The direct exposure of wild fowls' eggs can be increased, inter alia, by irregular use of the pesticides, by inappropriate spraying techniques and by side drifting of spray liquids (Várnagy and Budai, 1995). Pheasants are exposed to the effects of chemicals used in agriculture during egg laying and foraging, their reproductive period usually coincide with crop protection measures, which indicates the necessity of dealing with the harmful effects of pesticides on living organisms from ecotoxicological view of aspects (Köhler and Triebskorn, 2013). Ecotoxicological testing methods of pesticides focus on certain chemical agents mainly separately, but a fact cannot be neglected, that chemical loads present in a complex way thus joint toxic effects, interaction of simultaneously presenting chemical substances have to be considered where the components may modify the toxicity of each other (Oskarsson, 1983; Danielsson *et al*., 1984; Speijers and Speijers, 2004).

MATERIAL AND METHODS

For modelling the environmental cadmium load, 0.1% cadmium sulphate solution (Reanal-Ker Ltd., Hungary) (Safety datasheet, 2014) was used in individual and joint treatments. The herbicide Fozát 480 (480 g/l glyphosate [isopropylamine salt], Agro-Chemie, Budapest, Hungary) (Ocskó *et al.*, 2017) was used in individual and joint treatments in typical field application rate (2.0%). 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=35/group) was performed on the day of initiation of hatching. In the individual treatments, solutions and emulsions 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. For the preparation of solutions and emulsions 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 19th day of incubation. Within the framework of the pathological studies, the body weight of embryos, the number of dead embryos and the macroscopic malformations were determined and recorded. In case of the body weight data of live embryos, statistical comparisons among the groups were made with one-way analysis of variances. Because the results showed significant differences, Dunnett tests were also performed. In 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 8.82% (Table 1.). The rate of embryonic mortality could be considered sporadic which made it possible to use that group as a frame of reference. Malformation occurred in one case in that group (3.33%) (Table 2.). On the effect of the injected distilled water in the control group, the average body weight of embryos was 22.61 grams (Figure 1., 2.). On the effect of the 0.1% cadmium sulphate solution the embryonic mortality significantly increased ($p<0.001$) to 85.71% in comparison to the control group (Table 1.). There was not any malformation in this group (Table 2.). Injection of cadmium sulphate decreased the body weight of embryos (avg. 21.14 g) in comparison to the control group (22.61 g) (Figure 1.) but the difference could not be proved statistically. The treatment with the herbicide Fozát 480 (2.0%) caused 40.63% embryonic mortality (Table 1.). The increase in mortality proved to be significant ($p<0.01$) in comparison to the control group. There was not any malformation in the group treated with the herbicide (Table 2.). On the effect of the injection of the herbicide a significant decrease ($p<0.05$) in body weights (avg. 20.79 g) was revealed compared to the control (22.61 g) (Figure 1.).
The combined test with $CdSO_4$ and Fozát 480 herbicide resulted in a 100% embryonic mortality (Table 1., 2., Figure 1., 3., 4.).

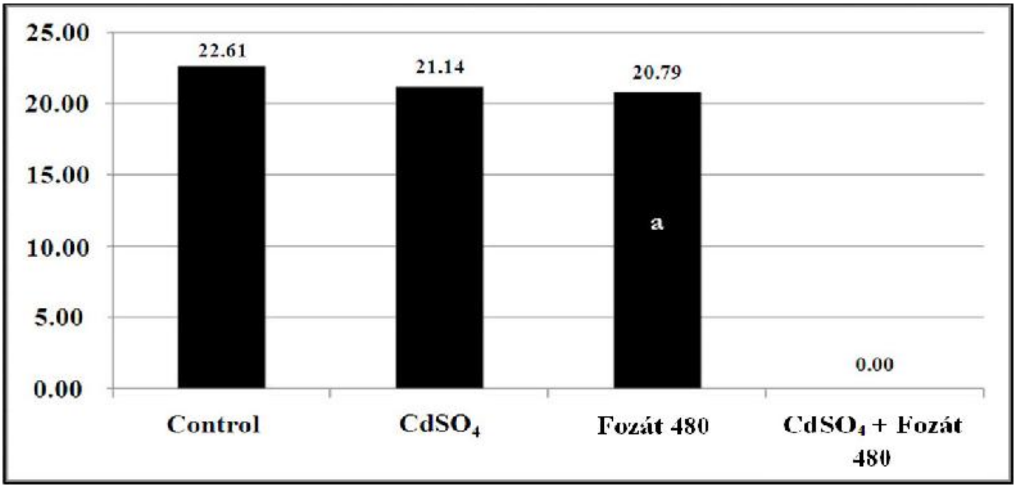
Table 1. The number and rate of dead embryos in the avian teratological test of individual and joint toxic effect of Fozát 480 (480 g/l glyphosate [isopropylamine salt]) and cadmium sulphate performed with injection treatment method.

Treated groups	**Number of dead embryos/ number of fertile eggs (pcs)**	**Rate of dead embryos (%)**
Control	3/34	8.82
Cadmium sulphate	30/35[a1]	85.71
Fozát 480	13/32[a2]	40.63
Cadmium sulphate + Fozát 480	34/34[a2]	100.00

[a]Significant difference compared to the control group ([a1] $p<0.05$, [a2] $p<0.001$).

Table 2. Number and rate of malformed embryos in the avian teratological test of individual and joint toxic effect of Fozát 480 (480 g/l glyphosate [isopropylamine salt]) and cadmium sulphate performed with injection treatment method.

Treated groups	**Number of malformed embryos/ number of alive embryos (pcs)**	**Rate of malformed embryos (%)**
Control	1/30	3.33
Cadmium sulphate	0/5	0.00
Fozát 480	0/19	0.00
Cadmium sulphate + Fozát 480	0/0	0.00



[a]Significant difference compared to the control group ([a]$p<0.05$).

Figure 1. Data of embryonic body weights in the avian teratological test of individual and joint toxic effect of Fozát 480 (480 g/l glyphosate [isopropylamine salt]) and cadmium sulphate performed with injection treatment method.

Figure 2. Chicken embryo on the 19th day of incubation

Figure 3. Mortified embryo on the 7th day of incubation

Figure 4. Mortified embryo with open chest cavity on the 18th day of incubation

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 increased the embryonic mortality. The herbicide Fozát 480 in individual and combined administrations significantly increased the embryonic mortality. The rate of malformations remained at a low level in all treated groups, so teratogenic effect could not be proved. Teratogenic effects of different doses

(500, 750 and 1000 mg/kg) of the herbicide Roundup (glyphosate) was investigated by Dallegrave *et al*. (2003) on pregnant rats. In their studies, it was revealed that different doses of the herbicide increased the embryonic mortality, which reached 50% at the highest dose. The frequency of embryonic skeletal malformations increased in the treated groups, which proved the teratogenic effect. The processing of results data showed a significant decrease in body weights in the treated groups compared to the control. In the group treated individually with 0.1% $CdSO_4$ the decrease in body weights was not significant, while 100% embryonic mortality was detected in the combined treated groups thus body weight data could not be measured.

CONCLUSION

Based on our avian teratological studies made with Fozát 480 and cadmium sulphate in individual and combined tests, it was established, that joint effects of both chemical agents in case of relatively low environmental cadmium load (which in itself could be less embryo toxic) the herbicide treatment with Fozát 480 in typical field rate additively increased the embryonic mortality, which represented in a significant decrease in bodyweights of embryos and in an increased embryonic mortality under the circumstances used in our experiments. According to research results, joint administration of pesticides generally increase moreover highly increase the toxicity of the chemical components multiplying the risk of their use as well. These effects are dependent on the species, time of exposition and doses so it is rather difficult to routinely forecast the expected effects (Thompson, 1996).

REFERENCES

Bogenfürst F. (2004). A keltetés kézikönyve. (Handbook of incubation.) Gazda Kiadó, Budapest. 42-63.

Danielsson B.R.G., Oskarsson A., Dencker L. (1984). Placental transfer and fetal distribution of lead in mice after treatment with dithiocarbamates. Arch. Toxicol., 55: 27-33.

Dallegrave E., Mantese F.D., Coelho R.S., Pereira J.D., Dalsenter P.R., Langeloh A. (2003). The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicology Letters, 142: 45-52.

Juhász É., Szabó R., Keserű M., Budai P., Várnagy L. (2006). Toxicity of a pendimethalin containing herbicide formulation and three heavy metals in chicken embryos. Comm. Appl. Biol. Sci., Ghent University, 71: 107-110.

Köhler H.R., Triebskorn R. (2013). Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341: 759-765.

Ocskó Z., Erdős Gy., Haller G., Molnár J., Eke I. (2017). Növényvédő szerek, termésnövelő anyagok (Pesticides and yield enhancing substances). Agrinex Bt., Budapest.

Oskarsson A. (1983). Redistribution and increased brain uptake of lead in rats after treatment with diethyldithiocarbamate. Arch. Toxicol., 6: 279-284.

Safety datasheet. (2014). 16231. Reanal Laborvegyszer Kereskedelmi Kft.

Speijers G.J. A., Speijers M.H.M. (2004). Combined toxic effects of mycotoxins. Toxicology Letters, 153: 91-98.

Szabó R., Budai P., Lehel J., Kormos É. (2011). Toxicity of s-metolachlor containing formulation and heavy metals to chicken embryos. Comm. Agr. Appl. Biol. Sci., Ghent University, 76: 931-938.

Thompson H.M. (1996). Interactions between pesticides; A review of reported effects and their implications for wildlife risk assessment. Ecotoxicology, 5: 59-81.

Várnagy L., Budai P. (1995). Agrárkémiai higiéne. (Agrochemical hygiene.) Mezőgazda Kiadó, Budapest. 45., 50-52., 64-65.