MODEL STUDY TO INVESTIGATE THE TOXIC INTERACTION BETWEEN KYLEO HERBICIDE AND LEAD ACETATE ON CHICKEN EMBRYOS

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Abstract

The aim of this study was to determine the individual and combined toxic effects of Kyleo herbicide (160 g/l 2,4-D; 240 g/l glyphosate) and lead acetate on the development of chicken embryos. The chicken eggs were dipped in the solution or emulsion of the test materials for 30 minutes before starting of incubation. The applied concentration of lead acetate was 0.01% and of herbicide Kyleo was 1%. The treatments were performed on day 0 of incubation, and the embryos were examined on day 19 by the followings: rate of embryo mortality, body weight, type of developmental anomalies by macroscopic examination. The body weight was evaluated statistically by one-way ANOVA with Tukey and Dunnett post-test, the mortality of embryo and the developmental anomalies were analysed by Fisher test. Lead acetate alone and in combination with herbicide significantly reduced the body weight of the embryos but statistically increased the mortality of embryo. Single and concomitant administration of lead acetate increased the rate of embryo mortality. Developmental abnormalities were observed sporadically due to the single and simultaneous administration of the test items. Based on the results there is a possibly additive toxic interaction between the lead acetate and Kyleo that can highly reduce the viability of the embryos or can lead to extinction of wild birds.

Keywords: chicken embryo, 2,4-D, glyphosate, lead acetate, toxic interaction, embryotoxicity

Összefoglalás

Napjainkban az emberiség egyik legnagyobb megoldásra váró problémája a természeti környezet növekvő mértékű elszennyeződése. A különböző vegyi anyagok egyidejűleg komoly kémiai terhelést jelenthetnek az egész élővilág számára, és mivel az interakciókra vonatkozóan csak kevés adat áll a rendelkezésünkre, ezért a legveszélyesebb nehézfémek körébe sorolt ólom, illetve egy széles körben alkalmazott herbicid, a glifozát és 2,4-D kombinált hatóanyagú Kyleo együttes méreghatásának tanulmányozását végeztük el. Arra kerestük a választ, hogy a környezetszennyezésből adódó állandó nehézfém-expozíció, kiegészítve egy gyakorlati permetlé töménységben alkalmazott Kyleo terheléssel; milyen változásokat indukál a tesztszervezetként választott házityúk-embrió fejlődésében. A kezeléseket a keltetést megelőző 0. napon végeztük el. Az embriókat vizsgálati anyagokból készült különböző koncentrációjú 38°C-os oldatba vagy emulzióba merítettük 30 perc kontakt időre. A kísérlet során az ólom-acetátot 0,01%-os, a herbicidet 1%-os koncentrációban alkalmaztuk. Az eredmények értékelése a keltetés 19. napján történt. A makroszkópos vizsgálat keretében lemértük az élő embriók testtömegét, feljegyeztük az elhullásokat és a fejlődési rendellenességeket és értékeltük azok gyakoriságát. A vizsgálati anyagokkal elvégzett egyedi és együttes kezelések eredményeként a gyomirtóval egyedileg kezelt csoporton kívül a kezelt csoportokban az embriók testtömeg értékei szignifikánsan kisebbek voltak a kontroll csoporthoz viszonyítva. Az egyedi és együttes kezelések következtében jelentkező elhullások száma - a herbiciddel egyedileg kezelt csoport kivételével - szignifikánsan emelkedett a kontroll csoporthoz képest. A fejlődési rendellenességek sporadikusan fordultak elő a kezelt csoportokban, teratogén hatás nem volt igazolható.

Kísérletünkben felhasznált 0,01%-os ólom-acetát oldat és Kyleo herbicid 1%-os emulziójának egyedi méreghatása embriótoxikus volt a tojásban fejlődő házityúk-embriókra. A kísérleti anyagok együttes alkalmazása során az embriótoxikus dózisú ólom-acetát mellett a növényvédelmi gyakorlatban felhasznált Kyleo gyomirtó szeres kezelés fokozta az embriótoxicitást, a toxikus interakció additív jellegű volt.

Introduction

The chemical plant protecting process is one of the most important polluting activities in the agricultural production. The ecosystem of a given habitat can be contaminated simultaneously by sprayed pesticides and other xenobiotics, e.g. heavy metals due to the agricultural activities during the plant protecting processes. Therefore, the chemical load can be occurred as a complex problem, so the combined toxic effect, i.e. toxic interaction of at least two substances can expected and the components can modify the effect of each other. Recently, the examination of the combination of heavy metals and other chemicals 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. Furthermore, the interaction effects are examined not only in the field of ecotoxicology, but also in all other areas that deal with health care and chemical safety issues (Oskarsson, 1983; Danielsson et al., 1984; Speijers and Speijers, 2004).

The different agricultural areas offer sources of food, shelter and breeding places to wild birds, therefore the sprayed pesticide and other chemical substances can contaminate not only the adults, but the embryos developing in egg, as well. The eggs of the wild birds may be exposed to different chemicals on the cultivated lands at the same time and their toxic effects may appear in embryo mortality and developmental anomalies.

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

The chicken embryo test is quick and accurate and allows the chemical impact on the embryo to be investigated. The further advantage of this method is its low cost, the sensitivity against various agents, as well as its high degree of similarity to the morphological development of mammals (Korhonen et. al. 1981, 1982). Chicken embryos is a proper model animal. Testing with it is providing the protection of wild bird population.

The aim of our study was to examine the toxic effect and interaction of lead acetate and glyphosate and 2,4-D containing herbicide (Kyleo) on chicken embryos after single and simultaneous administration of the test items by immersion technique.

Materials and Methods

Fertile chicken eggs of Farm breeds (Goldavis Ltd., Sármellék, Hungary) were used in the study. The eggs based on their size and weight, were divided into four homologue groups (40 eggs/group). The experimental design is presented in Table 1. They were incubated in a Ragus type table incubator (Wien, Austria, in 2018) assuring the required temperature (37-38°C), the relative humidity (50-65%) and the daily twice rotation of them to prevent the adhesion of the embryo to the egg-shell (Bogenfürst, 2004).

Table 1. Experimental Design

Group	**No of eggs**	**Treatment**	
		Lead acetate	**Kyleo**
I (control)	40	-	-
II	40	0.01%	-
III	40	-	1%
IV	40	0.01%	1%

Before starting of incubation the eggs were immersed for 30 minutes into a solution or emulsion with a temperature of 38ºC, that were prepared from the test items or from their combination. Then they were placed onto a filter paper after treatment to soak the unnecessary liquid.

During the single and simultaneous administration lead acetate (Reanal-Ker Ltd., Budapest) was applied with a concentration of 0.01%. The dose was selected based on previous studies to produce toxic effect alone but not to all treated eggs (Fejes, 2005). The 2,4-D and glyphosate containing herbicide, Kyleo (Nufarm Hungary Ltd., 160 g/l; 360 g/l) was used as 1% solution corresponding to that used in plant protection practice. The control group was treated with avian physiological saline solution (NaCl, 0.75 w/v%). The details of the experimental design are presented in *Table 1*. All eggs and embryos were examined and processed on day 19 of incubation. During the processing rate of embryo mortality, body weight of embryos and type of developmental anomalies were registered.

The distribution of body weight of the live embryos was controlled by Comparison-Quantile Plot and was analysed statistically by one-way ANOVA. Data of groups were compared by

Tukey and Dunnett tests. The statistical analysis of the results of embryo mortality and developmental abnormalities were performed by Fisher's exact test (Baráth et al., 1996).

Results

The average body weight of the embryos was 20.27±1.27 g in Group II that was significantly lower as compared to the control group (21.35±1.27 g; p=0.05). Due to Kyleo treatment the body weight was reduced to 20.72±1.57 g without statistical difference compared to the control.

The simultaneous administration of lead acetate and Kyleo resulted in significant decrease of average body weight (Group IV: 20.21±1.59 g; p=0.05) as compared to the control (p=0.05) (*Figure 1*).

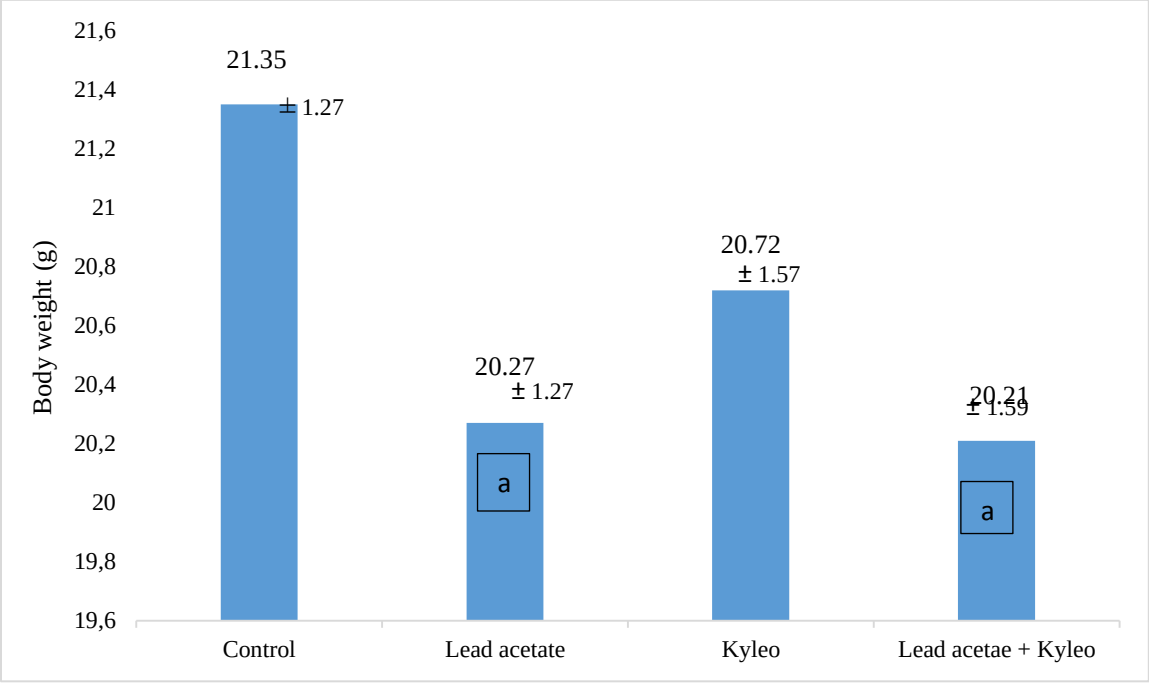

Figure 1. Body weight of the chicken embryos on day 19 of incubation from teratogenicity test on Kyleo and lead acetate after single and simultaneous administration (g)

a: Significant decrease as compared to the control(p=0.05)

The results of the embryo mortality and the developmental abnormalities are presented in *Table 2*.

There was no died embryos in the control group. The single administration of lead acetate increased the mortality up to 17.9% in Group II. The changes were statistically different (p=0.05) as compared to the Group I (control). The application of 1% Kyleo caused 5.1% of mortality in the treated embryos of Group III that was not significant as compared to the control. Due to the simultaneous administration of 0.01% lead acetate and 1% Kyleo (Group IV) induced significant increase (p=0.5) of embryo mortality (20.5%). Due to the simultaneous application of the test items the mortality rate of the embryos was increased up to 1.15 times higher compared to the Group II and 4 times higher compared to Group III. This increase is closely addition effect compared to the single treatments.

Table 2. Embryonic death and developmental anomalies from teratogenicity test of lead acetate and Kyleo in chicken embryos after single and combined administration

Group	Treatment	No of embryos showing abnormality/No of live embryos	Death No/ No fertile eggs	Rate of developmental anomalies (%)	Mortality (%)
I	Control	0/39	0/39	0	0
II	Lead acetate	2/39	7/39[a]	5.1	17.9
III	Kyleo	3/39	2/39	7.7	5.1
IV	Lead acetate + Kyleo	3/39	8/39[a]	7.7	20.5

a: Significant difference as compared to the control (p=0.05)

Developmental abnormalities were not recorded in the control group. The 0.01% lead acetate induced leg deformation and open abdomen (Group II: 2) without statistical difference as

compared to Group I. The applied concentration of Kyleo (1%) and lead acetate (0.01%) induced leg deformation, growth retardation and beak malformation.

Discussions

The single treatment of lead acetate with 0.01% concentration induced embryotoxic effect in chicken embryo that manifested in significant decrease of body weight and elevated rate of embryo mortality.

The results of the individual teratogenicity studies on lead acetate in chicken are in accordance with results of toxicity studies in other species. Depending on the dose, lead has embryotoxic potential and may cause developmental anomalies (Ferm and Carpenter, 1967; Várnagy and Budai, 1995). Similar results were found in chicken embryos treated with 0.01% lead acetate (lower body weight, higher rate of embryo mortality) but the developmental anomalies were not significant versus the control group (Juhász, 2009).

The glyphosate and 2,4-D containing Kyleo plant protection product with herbicidal action was also embryotoxic on chicken embryos and resulted in non-significant reduce of body weight and increase of mortality.

Glyphosate containing Roundup herbicide was examined by other researchers in Wistar rats. Dams were treated orally with 500, 750 and 1000 mg/kg glyphosate via drinking water. The results showed 50% mortality rate of dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in foetuses of the dams in groups treated with 500, 750 or 1000 mg/kg. Based on these data can be concluded that the glyphosate containing RoundUp is toxic to dams and induces developmental retardation of the foetal skeleton (Dallegrave et al., 2003).

Other studies reveal that acute treatment of male rats with 2,4-D caused reproductive system toxicity mainly with dose of 200 mg/kg (Marouani et al., 2016).

Due to the simultaneous application of lead acetate and Kyleo the embryo mortality was statistically higher than the individual effect.

Generally, the simultaneous application of heavy metals and pesticides may cause significant increase of their toxic effect in comparison with the individual toxicity of the applied components (Juhász, 2006).

According to the published literature the toxicity of many pesticide combinations is at least additive. In some cases pesticide mixtures, if they particularly contain insecticide component, have been shown to be synergistic effects, with reported increase in toxicity up to 100-fold. However, these effects are species, time and dose dependent, therefore difficult to predict it routinely (Thompson, 1996).

Acknowledgement

The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

References

Baráth, Cs., Itzés, A., Ugrósdy, Gy. (1996): Biometrics. Mezőgazda Publishing, Budapest. 37-217.

Bogenfürst F. (2004): A keltetés kézikönyve. Gazda Kiadó. Budapest. 42-63.

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. DOI: https://doi.org/10.1016/s0378-4274(02)00483-6

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. DOI: https://doi.org/10.1007/bf00316582

Fejes, S., Várnagy, L., Budai, P., Takács, I. (2001): A réz-szulfát és a BI 58 EC interakciós vizsgálata házityúk embrión. TOX'2001 Konferencia. Eger. Absztraktok C2–4.

Fejes, S. (2005): Egyes nehézfémek és növényvédő szerek egyedi és együttes méreghatásának vizsgálata madárteratológiai tesztben, Doktori Értekezés, Veszprémi Egyetem, Keszthely. 83-84.

Ferm, V.H., Carpenter, S.J. (1967): Developmental malformations resulting from the administration of lead salts. Experimental and Molecular Pathology. **7**: 208-213. DOI: https://doi.org/10.1016/0014-4800(67)90030-5

Institóris, L., Siroki, O., Dési, I. (2001): Kombinált cipermetrin Hg^{2+} és As^{3+} expozíció immuntoxikológiai vizsgálata patkányon. TOX'2001 Konferencia. Eger. Absztraktok C1–2. DOI: https://doi.org/10.1016/s1567-5769(01)00029-7

Kertész, V. (2001): Nehézfémek és PAH-vegyületek embrionális fejlődésre gyakorolt hatása madarakon. Doktori (PhD) értekezés. SZIE MKK. Gödöllő.

Korhonen, A., Hemminiki, K., Vainio, H. (1981): Application of the chicken embryo in testing for embryotoxicity. Thiurams. Scandinavian Journal of Work. Environment and Health, **8**: 63-69.

Korhonen, A., Hemminiki, K., Vainio, H. (1982): Embryotoxicity of industrial chemicals ont he chicken embryo: Thiourea derivatives. Acta Pharmacol. Toxicol., **51**: 38-44.

Juhász, É., Szabó, R., Keserű, M., Budai, P., Várnagy, L. (2006): Teratogenicity testing of a 2,4-D containing herbicide formulation and three heavy metals in chicken embryos. Commun. Agric. Appl. Biol., **71/2a**: 111-114.

Juhász, É. (2009): Herbicidek (Stomp 330 EC, Dikamin D) és nehézfémek (réz, kadmium, ólom) egyedi és együttes méreghatása madárembriókon. Doktori (PhD) értekezés. PE GK. Keszthely.

Marouani, N., Tebourbi, O., Cherif, D., Hallegue, D., Yacoubi, M.T., Sakly, M., Benkhalifa, M., Rhouma, K.B. (2016): Effects of oral administration of 2,4-dichorophenoxyacetic acid (2,4-D) on reproductive paramaters in male Wistar rats. Environmental Science Pollution Research. **24**: 519-526. DOI: https://doi.org/10.1007/s11356-016-7656-3

Oskarsson, A. (1983): Redistribution and increased brain uptake of lead in rats after treatment with dethyldithiobarbamate. Arch. Toxicol. **6:** 279-284. DOI: https://doi.org/10.1007/978-3-642-69083-9_52

Pecze, L., Papp, A., Nagymajtényi, L. (2001): Kombinált toxikus expozíció hatása az *in vivo* regisztrált hippokampális populációs spike-ra patkányban. TOX'2001 Konferencia. Eger. Absztraktok C1–4.

Speijers, G.J.A., Speijers, M.H.M. (2004): Combined toxic effects of mycotoxins. Toxicology Letters. **153:** 91-98. DOI: https://doi.org/10.1016/j.toxlet.2004.04.046

Thompson, H.M. (1996): Interaction between pesticides; A review of reported effects and their implications for wildlife risk assestment. Ecotoxicology. **5:** 59-81. DOI: https://doi.org/10.1007/bf00119047

Várnagy, L., Budai, P. (1995): Agrochemical hygiene. Mezőgazda Publishing, Budapest. 45., 50-52., 64-65., 70-83.

Várnagy, L., Varga, T., Hlubik I., Budai P., Molnár, E. (1996): Toxicity of the herbicides Flubalex, Fusilade S and Maloran 50 WP to chicken administration as single compounds or in combination. Acta Vet. Hung. **44:** 363-376. DOI: https://doi.org/10.1016/0378-4274(95)94972-j