THE INDIVIDUAL AND COMBINED TOXIC EFFECTS OF HERBICIDES (STOMP 330 EC, DIKAMIN D) AND HEAVY METALS (COPPER, CADMIUM AND LEAD) ON BIRD EMBRYOS

SUMMARY OF DOCTORAL THESIS

Written by:
ÉVA JUHÁSZ

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1. SCIENTIFIC ANTECEDENTS

We can say that in the past few years there has not been a month or sometimes a week without media news about environmental disasters. We could conclude that the cause of this is the faster flow of information. While earlier a letter arrived from a neighbouring country months after posting it, nowadays just click on the internet and you can read about all the events that have happened at different parts of the world. Although this statement is true it does not explain the rising number of environmental pollution and disasters in the news.

Of course if we talk about chemicals including herbicides we must not talk only about their harmful effects because if their application did not serve mankind, we would not have to deal with the topics of their use.

Nowadays it is not enough to produce the raw materials in big quantities to provide food for people, the production of safe and good quality food is getting more and more important. We all know that we need to use chemicals for this because if we think of the mycosis of plants and the dangerous toxins that appear there, we know that we can prevent the infections of our plants by using modern effective fungicides. We must think about the priority of the decrease of environmental pollution or food safety. In my opinion the answer is - as usual - the golden mean.

It is important to mention that the effective agents of herbicides do not cause problems only in extreme doses, the combined effects of smaller concentrations can also yield unexpected results. The number of possible interactions is infinite because a lot of chemicals can meet each other in the environment therefore the combined effects are incalculable with unforeseeable consequences. It is important to reveal the combined effects in the most comprehensive way even in the case of materials and concentrations which did not prove to be toxic individually during their examinations or licensing.

The population of the Earth is increasing year by year therefore a bigger amount and possibly good quality food is needed, which must be grown on a given extent of land, so results can be obtained only by increasing efficiency, because the amount of land is not infinite, we cannot increase it at will. The increase of efficiency means that we must be able to protect our plants from pests therefore we have no other choice but applying pesticides in agriculture and must devise new compounds, make their efficiency sure and reveal their possible harmful effects during licensing and in other cases as well.
In the previous years in Hungary – in accordance with the international practice – the toxic dangers of pesticides were determined by the acute LD50 values of the toxicological examinations of animal experiments. In the 1970s a new system was created to evaluate hazards, which ranks the pesticides into three categories of dangerousness (work, nutrition and environmental hygiene) taking six-six factors into consideration. Therefore instead of the previous one date dangerousness in each area of hygiene is evaluated by 18 factors because one date is not enough to be taken into account when possible harmful effects are evaluated but we must assess the toxic data of the chemical, its cumulative features, the circumstances of application and the possibilities of preventing intoxication and cure. (VÁRNAGY és BUDAI, 1995).

When pesticides are licensed the examination of the teratogenic effect became general of all necessary toxicological examinations at the end of the 1960s and the beginning of the 1970s (VÁRNAGY, 1991). In Hungary even the 5/1988 order of the Ministry of Agriculture and Food- taking the directives of the OECD about licensing chemicals into consideration – ordained the bird teratogenic examinations of hens and/or pheasants together with game toxicological examinations. Although the current regulation does not prescribe the teratogenic examinations of bird embryos these experiments still make the preliminary tests of medicine possible in order to evaluate teratogenic danger and they can be applied to examine other compounds from an eco-toxicological point of view especially in licensing pesticides and in the application in basic research (VÁRNAGY, 2005).
2. AIM OF RESEARCH

In this thesis the aim of my examinations was to reveal the individual and combined embryo destroying effect of heavy metals (copper, cadmium and lead), which are extremely dangerous for living organisms and occur in greater quantities in our environment and that of two widely applied herbicides (Stomp 330 EC, Dikamin D).

The examination is suitable to see how the toxic effects of the given pesticides are shown in the developing bird embryo during the whole phase of embryonic development. As the eco-toxicological methods of examination are mainly restricted to examine the toxic effects of one given material, the data regarding to the interactive effects – mainly in the organism of a developing bird – it can be considered as supplantory. This fact is especially important because the prior condition of revealing the interactions and the connections between the living creatures and the environment and evaluating them is to create a database with the exact data that support the result of examination.
3. MATERIALS AND METHODS

3.1. Material of examination

3.1.1. Herbicides

- Stomp 330 EC (33% pendimetalin)
- Dikamin D (72% 2.4-D)

3.1.2. Heavy metals

- Copper-sulphate
- Cadmium-sulphate
- Lead-acetate

3.2. Applied concentrations

In the case of heavy metals the applied concentration was 0.01% based on the results of a previous examination.

In my experiments I applied the herbicides in the concentration of normal sprays used in the work of chemical plant protection.

3.3. Experimental animals

In my experiments I used the eggs of hens (*Gallus gallus f. domestica*). During my examinations I used Shaver Rusticbro eggs, which were bought from the hatching plant of Goldavis Ltd. in Sármellék. The Shaver Rusticbro meat hybrid has favourable qualities and good yield capacity. During the series of experiment I used 1920 hen eggs altogether.
3.4. **Hatching**

I started the hatching of the eggs after a 24-hour rest following transportation in a Ragus type table hatcher. During hatching I provided the optimal temperature (37-38 °C) and humidity (65-70%) and the rotation of the eggs in order to avoid the sticking of the embryos.

3.5. **The method and type of treatment**

In my series of examinations I chose the start of hatching that is the 0 day, as the time of treatment. With this, I tried to model as if the chemical load affected the embryo at the beginning of development. Before starting the treatments I put the eggs into random groups trying to make homologous groups regarding the size and the weight, and I marked all the eggs at a distinctly visible place with a pencil.

In this thesis I examined the experimental materials with injection and dipping method. In the course of the injection treatment I drilled a hole through the shell above the air chamber then I introduced the solutions and emulsions of the experimental materials in suitable concentration into the air chambers of the eggs. Then I stamped the shell of the eggs with paraffin and put them into the hatcher. In the course of the individual treatments I introduced 0.1-0.1 ml of suitable concentration of the solutions of heavy metals and herbicides into the air chamber of the eggs while during the examination of the combined toxic effect 0.2 ml was injected altogether that is 0.1-0.1 ml of both experimental material. I used distilled water to make both the solutions and the emulsions and the eggs of the control group were injected with 0.1 ml of distilled water.

In the course of the dipping treatment I put the eggs into the solution or emulsion of the experimental material for 30 minutes, which had suitable concentration and was made with tap water of 37 °C. After finishing dipping I put the eggs on a filter paper to blot moisture, I marked them at a distinctly visible place with a pencil and put them into the hatcher. I used tap water of 37°C to make the solution of heavy metals and herbicides in suitable concentration and I dipped the eggs of the control group into tap water of 37°C for 30 minutes.
3.6. Processing

During my examinations the breaking and the processing of the hens’ eggs took place in two different periods. On the day of the start of hatching, in order to examine the phase of early development, I made permanent specimens on the 2\textsuperscript{nd} and 3\textsuperscript{rd} days of hatching, from 10 embryos of each group using 0.1% osmium-tetroxide paint (SINKOVITSNÉ és BENKÓ, 1993; KERTÉSZ, 2001). I broke the other treated eggs two days before hatching and I carried out the examination of the embryos from the following points of view:

- I weighed the body weight of the embryos, recorded the number and type of possible developmental disorders and the number and time of death.

- In order to carry out histological processing I took samples from the liver and the long neck muscle. From the organs, which were fixed in the 4% neutral diluted solution after embedding them in paraffin hematoxylin-eosin painting was carried out and the samples were evaluated using optical microscopes. (KRUTSAY, 1980; VETÉSI, 2002).

- I made a skeleton specimen by using alizarin – red paint in order to reveal the possible developmental disorders in the skeleton of the embryos (DAWSON, 1926), the evaluation was made by stereo-microscope.

3.7. Statistical examination

I carried out Kolmogorov – Smirnov test to examine the distribution of the data of body weight, then as the data had normal distribution, Student type t- test was applied (FINNEY, 1972). The statistical evaluation of embryo mortality and developmental disorders was done by RXC Chi\textsuperscript{2} - test. (BARÁTH és mtsai. 1996).
4. RESULTS OF EXPERIMENT AND EVALUATION

4.1. Early development of embryos

During the early stage of the development of embryos I concluded that heavy metals intensified the number of death when injection treatment was carried out but I did not find significant increase using copper, cadmium-sulphate, or lead-acetate in the examined parameters and it was justified by the results of other authors (FEJES, 2005). In this phase for the development of hen embryos Stomp 330 EC in the concentration of normal spray was the most toxic of the pesticides and heavy metals that were introduced into the egg individually in the case of both methods of treatments. None of the individual treatments resulted in significant deviation comparing them to the control group.

Of the groups which had combined treatment the injection application of Dikamin D herbicide combined with any heavy metals and Stomp 330 EC combined with lead-acetate caused the most significant number of embryo deaths comparing it to the control group.

We can conclude that in each treated group embryos with developmental disorders occurred sporadically I did not experience significant deviation. Developmental disorders appeared in the underdevelopment of the venous network, the somites and in the deviant development of the brain bladder and the eye bladder.

4.2. Pathological processing

During the processing on the nineteenth day, as a result of injection treatments, cadmium-sulphate and lead-acetate increased the rate of death significantly in comparison with the control group moreover the three heavy metals decreased the body weight of the animals significantly in the case of both methods of treatments in comparison with the control group.

In the groups, which were treated by herbicides individually the rate of dead embryos increased significantly in comparison to the values of the control group during the injection treatments. Similarly to the results experienced in the examination of the early developmental
phase, the embryo toxic effect of the products occurred right after the period of hatching in the case of both pesticides.

In the groups treated individually the frequency of developmental disorders occurred significantly in the case of copper-sulphate and Dikamin D, and in the case of copper and cadmium-sulphate during the dipping treatment in comparison to the control group.

As the result of the combined treatments, during the injection treatment, the number of embryo deaths and the frequency of developmental disorders increased significantly in comparison to the control group. Stomp 330 EC combined with any heavy metal increased the number of embryo deaths significantly, even in comparison with groups treated with herbicides and heavy metals individually, while Dikamin D caused the significant increase of deaths only with the combined application with cadmium-sulphate in comparison with the individually treated groups.

In comparison to the individually treated groups, during injection the frequency of developmental disorders increased significantly when the Stomp 330 EC, copper-sulphate and lead-acetate were applied together, while in the course of dipping the combined application of Dikamin D and lead-acetate caused the significant increase of developmental disorders in comparison to the groups treated with heavy metals individually.

As a result of combined treatments both methods of treatments caused a significant decrease of body weight in comparison to the control group. During injection the herbicide Dikamin D caused significant loss of body weight combined with any heavy metal in comparison to the groups treated with heavy metals and herbicides individually.

4.3. Processing with skeleton painting

Of the chemicals applied individually the use of copper-sulphate and Dikamin D caused the highest rate of developmental deviations, which occurred as the faulty posture of the legs and neck and the retardation of growth.

In the case of the groups which had combined treatment the use of Dikamin D together with any heavy metal increased the developmental disorders which could be detected by Dawson-type painting technology in comparison to the groups treated individually. The deviations were the faulty posture of the legs and neck and the retardation of growth.
4.4. Histological processing

The examination of the pathological histology of the treated animals did not show any liver, heart or muscle damaging effects of the applied chemical compounds in any of the examined groups.

4.5. Suggestions

- We should examine chemicals, which are used widely, how the given chemical acts together with other chemical materials which are present in nature or in the organisms of animals. Therefore I suggest widening interactive examinations regarding other widely used chemicals.

- The experiments I carried out prove clearly that the examination of early development can be a good method of pre-examination during the licensing process because the effects of the heavy metals and herbicides I examined can be well seen in the early developmental phase of the embryos, which is proved by the result of process of the 19th day.

- I suggest repeating the experiment with a spraying method as well because the expositional circumstances that really occurs in nature can be modelled with this method more precisely, and we could get important data how the much and what form the chemicals examined in this method of examination can present their influences when a third method of treatment is applied.

- The experiments I carried out can base the evaluation of the toxic dangers wild birds but due to the sensitiveness of wild birds this experiments should be repeated on pheasants, wild ducks and Japanese quails.

- The examination I did could be completed with the determination of the parameters of the embryos’ blood plasma, which could provide a more precise data about the harmful effects of the chemicals applied in the experiment on living organisms.
• The experiment I did on the examined pesticides could be completed with an examination of disintegration dynamics, which could show the behaviour of herbicides in the organisms of animals more precisely.

All in all we can conclude that the introduction of the suggested new methods and procedures and the expansion of the examination of materials which are harmful to the environment we can learn more about the influence of man on wildlife and using the results in practice we have a better chance to preserve our environment in its original state for our grandchildren.
5. New scientific results

1. From the data that I gained after processing them on the 19th day I concluded that in the groups that were treated with 0.01% copper-sulphate, Dikamin D applied in the concentration of normal spray and Stomp 330 EC, the chemicals had toxic effects on embryos, in comparison with the control group, their harmful effects could be observed as significant weight loss in the case of both methods of treatments. During the injecting treatment deaths increased significantly in comparison with the control group and the group treated with heavy metals individually.

2. After the data processed on the 19th day I concluded that the joint use of the 0.01% concentration of cadmium sulphate, the Dikamin D applied in the concentration of normal spray and Stomp 330 EC herbicide had toxic effects on embryos, in comparison with the control group, their harmful effects could be observed as significant weight loss mainly in the case injection treatments. Deaths of embryos increased significantly in comparison with the control group and to the group treated individually.

3. After the data processed on the 19th day I concluded that the joint use of the 0.01% concentration of lead acetate the Dikamin D applied in the concentration of normal spray and Stomp 330 EC had toxic effects on embryos, comparing them to the control group, their harmful effects could be observed as significant weight loss in comparison with the control group. As the effect of injecting the frequency of development disorders and the deaths of the embryos increased significantly in comparison with the control group.

4. After comparing the two methods of treatment, I concluded that during the examination of the two kinds of herbicide (Dikamin D, Stomp 330 EC) in the concentration of normal spray and the three types of heavy metal( copper-sulphate, cadmium-sulphate and lead-acetate) in 0.01% concentration, the injection treatment proved to be more toxic in comparison with dipping, which appeared in the higher increase of development disorders, and the deaths of embryos.
6. PUBLICATIONS

Scientific publications on the subject of the thesis

Scientific articles


Other publications


