The use of parasites as bioindicators of pesticide exposure

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ABSTRACT

Organisms used in risk assessment of pesticides must be the most sensitive ones to pesticides exposure. The aim of this study was to observe the effect of two commercial pesticide products (containing glyphosate and tolylfluanid) to larval stages of parasites Cooperia curticei, Ostertagia circumcincta, Haemonchus contortus and Trichostrongylus axei. There were two concentrations tested for each product vs. control group. Larvae (500 individuals/Petri dish) were incubated at 27 °C and observed daily for 42 days. We found out that T. axei larvae are the most resistant ones to tolylfluanid exposure – there was no statistical significance in any concentration tested after 42 days of tolylfluanid exposure. 100% of dead larvae were found on 33rd day of experiment at higher concentration, resp. on 37th day at lower tested concentration of glyphosate. C. curticei, O. circumcincta and H. contortus showed similar statistical significance in both pesticides tested (there was high statistical significance (p<0.0001) at both concentrations of glyphosate and only at higher tested concentration of tolylfluanid). C. curticei and H. contortus larvae were found dead, spiral shaped and without movement at all concentrations tested, spiral shape was not observed in other two tested larvae. O. circumcincta larvae reacted to pesticides exposure very quickly; rapid death was recorded on second day of experiment at both concentrations of glyphosate and at higher tested concentration of tolylfluanid. From four tested small ruminant parasites (L3), O. circumcincta larvae seem to be the most sensitive ones and need further research.

KEY WORDS: pesticides; parasites; exposure; bioindicators

Introduction

Pesticides are frequently used in commercial agriculture to increase production despite their proven negative side effects on consumer health. The very significant impact of European legislation on the authorization of plant protection products (Directive 91/414/EEC) has resulted in withdrawal of 704 out of total 889 active substances assessed so far. There was observed at least one health side-effect including carcinogenicity, reproductive and neuro-developmental disorders, as well as endocrine disruption after acute or chronic exposure of 84 approved active substances (Karabelas et al., 2009).

All relevant living forms (birds and mammals, aquatic, bees and non-target arthropods, soil micro- and macro-organisms, non-target plants and sewage treatment) are included in the risk assessment of pesticides (it is ecotox section in Draft Assessment Report prepared by Notifier and reviewed by EFSA). Parasites and their development stages, frequently present in animal dung, get to the environment (e.g., surface layer of soil) by manuring and can be easily exposed to pesticides applied on crops. That is why the aim of this study was to observe the in vitro effect of two commercial pesticide products (containing glyphosate, resp. tolylfluanid) on larval stages of four common small ruminant parasite species. According to obtained results to assess whether the tested parasites are suitable for the risk assessment of pesticides and/or chemical substances.

The experiment included Cooperia curticei, Ostertagia circumcincta, Haemonchus contortus and Trichostrongylus axei. Product, containing glyphosate, is used as herbicide and second commercial product, containing tolylfluanid, was used as fungicide. The use of Plant Protection Products (PPP) containing active substance tolylfluanid is nowadays prohibited in Europe on the basis of commission restriction Nr. 2007/322/EC (Official Journal of the European Union).

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Materials and methods

Chemicals
In our experiment we used following chemical substances: Herbicide Roundup Rapid® (Monsanto Europe S.A., Belgium) with the active substance glyphosate 450 g/L product and fungicide Euparen Multi® (Bayer AG, Germany) with the active substance tolylfluanid 500 g/L product.

There were two concentrations tested for each product (registered maximal concentration of active substance per ha and 10 times higher concentration) vs. control group (distilled water) in this study. In the case of glyphosate, we used concentrations of 1.4% and 14% and in the case of tolylfluanid it was 0.25% and 2.5% concentration.

Parasite cultures
Larvae (L3) of Cooperia curticei, Ostertagia circumcincta, Haemonchus contortus and Trichostrongylus axei were obtained from Veterinary Laboratories Agency (Weybridge, UK). They were stored under the temperature of 4 °C until the use.

Method of testing
Total amount of 500 larvae individuals were dosed with automatic micropipette per each tested concentration to Petri dishes (diameter of 40 mm). The number of larvae was microscopically calculated and if needed, (when number of individuals per a Petri dish was not reached) larvae were dosed again to reach correct value.

After application of pesticide concentrations (distilled water in control group), Petri dishes were incubated at average temperature of 27 °C in laboratory incubator (TER 80). Effects were regularly observed by using microscope (Nikon) every day for 42 days. If the effect of pesticides was present, larvae were found dead, without movement after microscope lighten.

Data analysis
Results were statistically evaluated by using the Contingency table (GraphPad Prism® 3), where \( p<0.05 \) was regarded as significant.

Results
From four tested ruminant parasites, T. axei larvae were the most resistant ones to tolylfluanid exposure – no statistical significance was observed in any tested concentration of tolylfluanid after 42 days of exposure (\( p=1.000 \)). In the case of glyphosate, 100% of dead larvae were found on 33rd day of experiment at higher concentration, resp. on 37th day at lower tested concentration.

Survival of C. curticei, O. circumcincta and H. contortus showed similar statistical significance in both pesticides tested (experiment vs. control groups), where high statistical significance (\( p<0.0001 \)) was observed in both concentrations of glyphosate and only at higher tested concentration of tolylfluanid.

Larvae of O. circumcincta reacted to pesticides exposure very fast; rapid loss of live larvae (\( p<0.001 \)) was recorded on 2nd day of experiment at both concentrations of glyphosate and at higher tested concentration of tolylfluanid.

Larvae of C. curticei were found dead (\( p<0.001 \)) on 22nd day of experiment at 1.4% glyphosate and on 19th day at 14% glyphosate. Both concentrations of glyphosate were significantly toxic to H. contortus larvae on 5th day of incubation (100% of larvae were found dead).

Tolylfluanid was significantly toxic to larvae of C. curticei and H. contortus (\( p<0.001 \)) only at 2.5% concentration after 42 days of exposure.

Dead larvae of C. curticei and H. contortus were spiral shaped and without movement at both products tested (Figure 1), while spiral shape was not observed in other two larvae tested.

Figure 1. Spiral shape of the dead larvae of Haemonchus contortus.
Discussion

Chemical substances get to the environment by human activity in different ways. The most dangerous are those with long persistency and cumulative properties (Legáth et al., 1997). Parasites circulate in the environment among intermediate and definitive hosts (Ciberej et al., 2001), so it means they are exposed to environmental pollution (Hronec et al., 2002). Parasites may be exposed in external environmental conditions (e.g., egg stages on the field) or in the animal body (intake of contaminated food and water).

Parasites react to chemical exposure in different ways. There was observed low prevalence of small ruminant gastrointestinal nematodes (larval stages) on the pastures contaminated with heavy metals (mercury dominated) in Spiš region (Krupicer, 1995). Papajová (2001) observed significant in vitro ovocidal effect of several disinfectants approved for use in farms in Slovakia against A. suum. Legáth et al. (2005) found out that tolylfluanid was in vitro very effective against coccidial oocysts in concentration range 0.001–1.0 g/L after 28 days of exposure.

In our experiment, glyphosate was more toxic to parasite larvae than tolylfluanid. From four tested ruminant parasites (L3), larvae of O. circumcincta were the most sensitive and larvae of T. axei were the most resistant ones after 42 days of both pesticides exposure.

These results are new and there is a lack of information about the effect of pesticides on animal endoparasites. That is why there is a problem to discuss them with similar ones.

Investigation of the influence of several chemical substances (including glyphosate) on protozoan parasite Perkinsus olseii proliferation revealed that glyphosate inhibited in vitro proliferation of this parasite in a dose-dependent manner (Elandalloussi et al., 2005). Another experiment in which horsehair worms Chordodes nobilii (Gordiida, Nematomorpha) were exposed to glyphosate concentrations ranging between 0.1 and 8 mg/L for a short period of time detected that embryo development was not inhibited, but there was a significant decrease in the infective capacity of larvae derived from eggs that had been exposed to concentration ≥0.1 mg/L. Adult exposed to 1.76 mg/L of formulated glyphosate for 96 h shown a mortality of 50% (Achiorno et al., 2008)

Our results similarly to those of Legáth et al., Elandalloussi et al. and Achiorno et al. point out that the pesticides like glyphosate and tolylfluanid have some antiparasitic effect in vitro although they are primarily herbicides, resp. fungicides.

This study was focused on parasite larvae stage with assumption they are more sensitive to exposure than other life stages (Borošková and Dvorožnáková, 1997; Papajová, 2001). It is necessary to continue in survey of all metamorphosis phases of the most sensitive parasite species and their infective capacity. On the basis of complex research, the most sensitive parasite species with their stages could be set for risk assessment of different chemical compounds.

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REFERENCES

Achiorno CL, Villalobos C, Ferrari L. (2008). Toxicity of the herbicide glyphosate to Chordodes nobilii (Gordiida, Nematomorpha). Chemosphere 71: 1816–1823.

Borošková Z. and Dvorožnáková E. (1997). The effect of cadmium on the immune behaviour of guinea pigs with experimental ascariasis. J Helminthol 71: 139–146.

Ciberej J. (2001). Ekológia, in Starostlivosť o zver a choroby zveri (Ciberej J ed) pp. 7–13, Par PRESS s. r.o., Bratislava.

Elandalloussi LM, Rodrigues PM, Alfonso R, Leite RB, Nunes PA, Cancela ML. (2005). Shikimate and folate pathways in the protozoan parasite, Perkinsus olseii. Mol Biochem Parasitol 142, 106–109.

Hronec O., Tóth J. and Tomáš J. (2002). Cudzorodé látky a ich riziká. Harlequin Ltd., Košice, ISBN 80-968824-0-6, 200 pp.

Karabelas AJ, Plakas KV, Solomou ES, Drossou V, Sarigiannis DA. (2009). Impact of European legislation on marketed pesticides – a view from the standpoint of health impact assessment studies. Environ int 35: 1096–1107.

Legáth J, Blíha K, Čermáková T, Dolináy S, Huňagelová B, Kočičová A, Kolluth P, Kotleba J, Legáth E, Mazáčová L, Markovič L, Mlynárčíková H, Mutín M, Ondráľovčíková M, Prošáková M, Škulínková K, Sokol J, Toporčák J. (1997). Odbd miery rizika chemických látok pre domáce, hospodárske a voľne žijúce zvieratá, včas a vodné živočíchy. DATAHELP, Košice, ISBN 80-88867-10-X, 102 pp.

Legáth J, Vasilková Z, Krupicer I, Sabo R. (2005). Členy fúnkigátu tolylfluanidu a herbicídù benzetáznu na atenuované oocysty kókoidí. Status Veterinarius 139–146.

Krupicer I. (1995). Vplyv imisí o šťahých kovov s dominanciou otrúti na priebeh pasienkových helmintóz oviec. Veterinární Medicína 40: 11–15.

Official Journal of the European Union [webpage on the internet]. COMMISION DECISION of 4 May 2007 laying down protective measures concerning uses of plant protection products containing tolylfluanid leading to the contamination of drinking water (notified under document number C(2007) 1865) (Text with EEA relevance) (2007/322/EC) [published 2007 May 9]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:19:0049:0050:EN:PDF

Papajová I. (2001). Pôsobenie vybraných abiotických a biotických faktorov pros-tredia na tenacitu vajíčok modelového nematóda Ascaris suum. Thesis (Dissertation): 196 pp.