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Toxicological effects of technical grade and formulated pesticides on esterase activity in freshwater snails *H. duryi and L. natalensis*

Norah Basopo*[1] and Yogeshkumar S. Naik[2]

Department of [1]Applied Biology and Biochemistry and [2]Environmental Sciences and Health, National University of Science and Technology, Ascot, Bulawayo

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ABSTRACT

We investigated the effects of formulated pesticides on non target organisms in order to evaluate the role played by inert ingredients in the toxicity of agrochemicals. The effects of both technical grade and formulated pesticides on esterase activity of two freshwater snail species Helisoma duryi and Lymnaea natalensis was investigated. Snails from both snail species were exposed to chlorpyrifos, dimethoate, carbaryl, lambda cyhalothrin, deltamethrin or mancozeb for 72 hours. Some of the snails were exposed to technical pesticides while others were exposed to the commercial forms of the pesticides. After the exposure duration post-mitochondrial supernatants of whole snail homogenates were prepared and used to measure choline and non-cholinesterase activity using substrates, acetylthiocholine iodide or α-naphthyl acetate respectively. The results showed that both formulated and technical grade pesticides significantly inhibited esterase activity. The inhibition observed in snails exposed to formulated commercial pesticides was twofold or more when compared to inhibition observed in snails exposed to technical grade pesticides and this was attributed to presence of inert ingredients in formulated pesticides. The enhanced inhibition of esterase activity observed in snails exposed to formulated pesticides indicates that aquatic organisms are potentially at risk to the toxic effects of the unspecified ingredients which are part of formulated pesticides.

Key words: Freshwater snails, formulated pesticides, technical grade pesticides, esterases.

INTRODUCTION

Technical pesticides also referred as pesticide standards are made up of only the active pesticidal chemical compounds. Formulated pesticides on the other hand are composed of two parts, namely; the active and inert ingredients. Active ingredients are chemicals which actually control and/or kill the pests. On the other hand inert ingredients are the carrier or sticking agent in the effective pesticide product. They may be solvents, stabilizers, preservatives, surfactants, sticking or spreading agents, or defoamers [1]. Most synthetic pesticides lack persistent residual action and are thus associated with frequent applications for effective results; this extensive use of formulated agrochemicals may present risks to natural aquatic ecosystems which eventually receive these chemicals.

Normally knowledge on the toxicity of most crop protection chemicals is obtained from exposure studies done using the active ingredients/standards of these agrochemicals [2; 3]. However, the few studies available in literature indicate that the bulk of pesticide formulations are actually the inert ingredients [4]. Some of the "inert" ingredients have been shown to be equally or more toxic than the active ingredient and/or may be an active ingredient in other pesticide products [4].

Most of laboratory-based studies on the toxicity of agrochemicals to living organisms are done using technical grade pesticides and very little information is available on the toxicity of the inert ingredients that make up the commercial formulated pesticides used by farmers and in industry. This observation is collaborated by [5] who stated that information on the toxicity of pesticides is normally provided for active ingredients rather than the formulated product. The two authors also alluded to the limited data regarding the toxicological impact of pesticides under natural or field conditions. In light of the fact that farmers and /or industry use formulated pesticidal products, there is need to assess and evaluate the toxicity of commercial pesticides in order to fully appreciate the toxic effects of pesticides. The objective of this study was to investigate *in-vivo* effects of several popularly used pesticides in Zimbabwe as technical grade (standard) chemicals and in their commercial formulated forms on esterase activity of two aquatic snail species *Helisoma duryi* and *Lymnaea natalensis* with the purpose of assessing the potential toxicological effects of inert components of formulated pesticides on aquatic life. Activities of esterases were measured as endpoint parameters because altered choline and non-cholinesterase activities of different aquatic organisms have been shown to be reliable biomarkers of exposure to agrochemicals [6; 7; 8].

MATERIALS AND METHODS

Chemicals

All the pesticides, substrates and standards were bought from Sigma Chemical Company, Germany. All other laboratory reagents were of analytical grade.

Snail breeding and exposure

Two species of snails, *Helisoma duryi* and *Lymnaea natalensis* were bred in cement tanks containing tap water and were fed on fresh garden lettuce according the method of [9]. Groups of adult snails (20) were exposed to 25 ppb of carbaryl, mancozeb, chlorpyrifos, dimethoate, lambda cyhalothrin and deltamethrin as formulated and technical grade pesticides for 3 days. The concentrations of the pesticides used in the present study were adopted from the literature and they have environmental significance as they have reported in the field [10; 11]. The exposures were performed in quadruplicate bowls with food, water and pesticide refreshed every 24 hours. After the exposure period post-mitochondrial fractions were prepared.

Post mitochondrial fraction preparation.

Snails from each group were washed once with tap water to remove leaf particles and other dirt. The snails were deshelled, pooled and homogenized, with ice-cold homogenization buffer, 0.1 M potassium phosphate pH 7.4 using a glass teflon homogenizer. The volume of the buffer used was equivalent to 3 times the weight of soft tissue of the snails. The whole organism homogenates were centrifuged at 10 000 *g* for 10 minutes using a Juan refrigerated high-speed centrifuge and the resultant supernatant fraction referred to as the post mitochondrial fraction (PMF), stored at -80^{o} C until required for the enzymatic assays.

Protein determination

Protein concentration was measured following method of [12] and bovine serum albumin was used as the standard.

Assessment of esterase activity

The PMFs were used as the enzyme source in the determination of esterase activity.

Non-cholinesterase activity

Non-cholinesterase activity was measured using the substrate α-naphthyl acetate following the method of [13]. The reaction mixture contained 4 mL of reagent A (reagent A contained a mixture of 1 mL of 25 mg/ml α-naphthyl acetate plus 100 mL of 50 mg Fast Blue RR salt dissolved in 0.1 M Tris/HCl buffer pH 7.4) and 20 µL of 0.1 mg/mL PMF. The mixture was incubated for 10 minutes in the dark. The reaction was stopped by addition of 1 mL of 20% (v/v) acetic acid and absorbance was measured at 605 nm against an appropriate blank.

Cholinesterase activity

Cholinesterase activity was measured using the substrate acetylthiocholine iodide according to the method described by [14]. The reaction mixture contained 50 µL of 0.1 mg/mL PMF, 110 µL of 0.01 M Tris/HCl buffer pH 8.0 and 50 µl of 0.4 mM 5,5 dithio-bis-(2 nitro benzoic acid) DTNB. The mixture was incubated for 3 minutes before adding 30

μL of 0.5 mM acetylthiocholine iodide. The rate of production of a complex between thiocholine and DTNB was followed for 3 minutes at 412 nm using a SpectraMax 340pc plate reader.

RESULTS

All pesticide standards and formulated products significantly decreased carboxylsterase activity ($p<0.05$, $p<0.01$ or $p<0.001$) in whole tissues homogenates of freshwater snails *H. duryi* and *L. natalensis*. Pesticide standards caused inhibition of carboxylesterase activity in the range 13-36% while pesticide formulation caused inhibition in the range 47-81% depending on the pesticide and snail species.

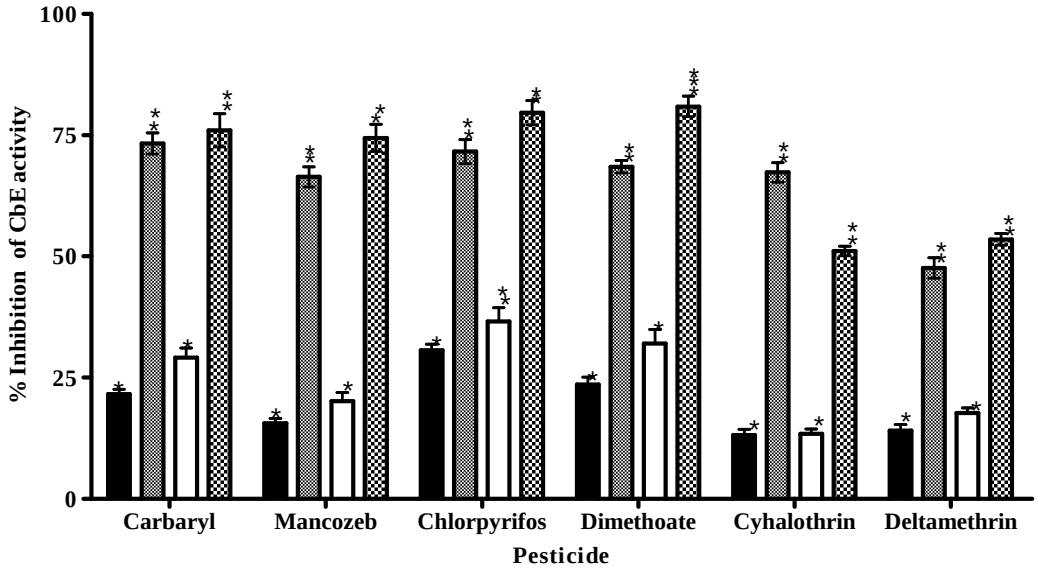

Figure 1. Effect of carbaryl, mancozeb, chloropyrifos, dimethoate, lamda cyhalothrin and deltamethrin as technical standards (*H. duryi* –standard), *L. natalensis* - standard) and commercial formulations (*H. duryi*-formulation), (*natalensis*-formulation) on cholinesterase activity in the freshwater snails *Helisoma duryi* and *Lymnaea natalensis*. Esterase activity was measured using α-naphthyl acetate as the substrate. Values represent % activity of controls obtained from the average of quadruplicate exposures (each containing pooled samples of 20 snails) and mean + SD of specific activity values. Significantly different from controls at (*P<0.05 **P<0.01 or ***P<0.001).

Cholinesterase activity was inhibited in the range 18-38% by pesticide standards while pesticide formulations caused inhibition in the range 45-94% depending on the pesticide and snail species. For all pesticides, the commercial pesticide formulations caused inhibition of esterase activity that was greater or equal to double the inhibition of esterase activity caused by corresponding technical pesticide standards.

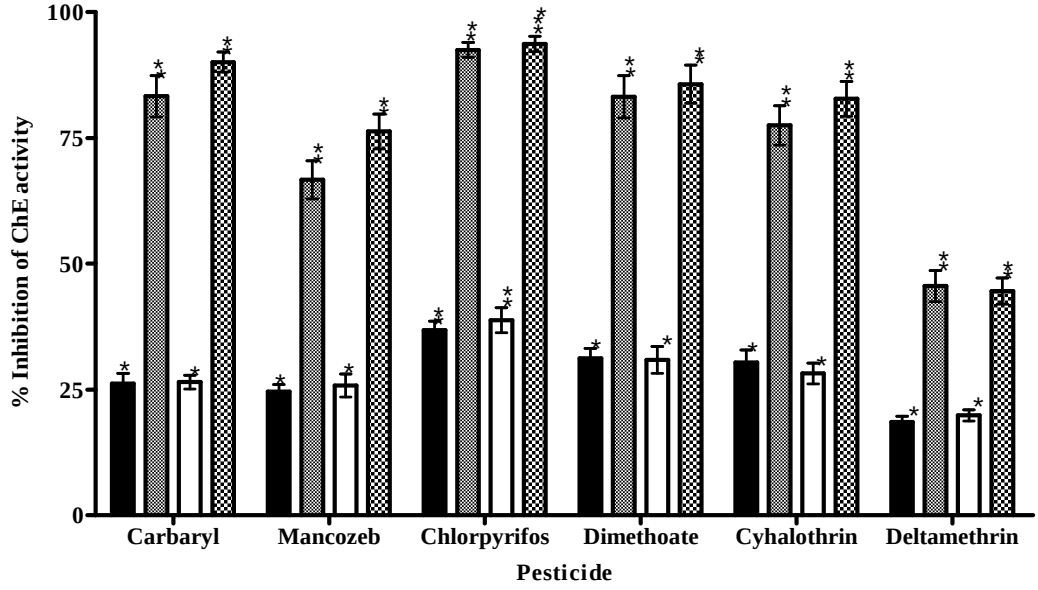

Figure 2. Effect of carbaryl, mancozeb, chloropyrifos, dimethoate, lambda cyhalothrin and deltamethrin as technical standards (*H. duryi* –standard, *L. natalensis*- standard) and commercial formulations (*H. duryi*-formulation), (*L. natalensis*-formulation) on cholinesterase activity in the freshwater snails *Helisoma duryi* and *Lymnaea natalensis*. Esterase activity was measured using α-naphthyl acetate as the substrate. Values represent % activity of controls obtained from the average of quadruplicate exposures (each containing pooled samples of 20 snails) and mean + SD of specific activity values. Significantly different from controls at (*P<0.05 **P<0.01 or ***P<0.001).

DISCUSSION

All pesticides both technical and formulated products caused significant reduction of both carboxylesterase and cholinesterase activities in the two freshwater snail species. The inhibitory effects of formulated products were up to 5 fold higher than the inhibitions caused by the identical concentrations of the same pesticides in their technical forms. Highlighting, technical grade mancozeb for instance, this fungicide exhibited low carboxylesterase inhibitory effects, however, the inhibitory effects of formulated fungicide, the form used by farmers, its inhibitory effects superseded those of the technical form of the pesticide by more than fourfold in both snail species. Higher toxicological effects of formulated pesticides when compared to the effects of the same concentrations of the technical form of the pesticide has been reported in other aquatic organisms [15; 16].[17] also reported higher toxic effects of formulated forms of chlorpyrifos and mancozeb when compared to effects of the technical form of the same pesticides on reproduction, growth and survival of the tropical earthworm *Perionyx excavates.* Since the difference between technical and formulated pesticides is the presence of inert material in the formulated pesticides was is absent in technical grade pesticide, any difference between the effects of the two forms of pesticides is attributed to presence of inert ingredients in pesticide formulations. The higher inhibitory effects of all the formulated pesticides used in the present study when compared to the effects of the technical form of the pesticide, is therefore, attributed to the inert constituents of the formulated pesticides. The sharp decreases in esterase activity observed in snails exposed to pesticide formulations is a cause for concern considering that, it is the pesticide formulation that is used in agriculture and in public health.

Literature reports indicate that only small quantities of the applied pesticide chemical reach the target organism, sometimes as low as only 0.03% [18]. The major portions of these chemicals end up in aquatic or other terrestrial ecosystems where they affect non target organisms. Pesticide labels show that the bulk of a pesticide formulation is the inert component and quite often constituting as much as 95% of the commercial product [4]. Duragon 8 Dust, a popular commercial product worldwide, is used to control aphids, in several crops including maize, groundnuts, potatoes, wheat, sorghum and tobacco contains 8% dimethoate and 92% inert ingredients [19]. These inert components are however, not specified. It has been reported in literature that despite the term “inert,” these ingredients may not be chemically, biologically or toxicologically inert [20] In fact, “inert” ingredients can be more

toxic than the active ingredient and in some cases are even used as active ingredients in other pesticide products [17]. This is confirmed by [4], who reported a study that revealed that from a 1ist of 1 995 inert ingredients surveyed, 394 of these chemicals were listed as active ingredients in other pesticide products.

A few studies have investigated the toxicological effects of formulated pesticide products on aquatic biota [21]. [22] showed that commercial formulations of the pesticides: diazinon, carbaryl, malathion and glyphosate reduced larval growth and survival in a dose response manner in five amphibian species. In another study, [23] reported that both muscle and eye ChE activities were greatly reduced in white shrimp (*Litopenaeus vannamei*) exposed to Tamaron 600 a commercial grade of methamidophos. The pesticide formulations also caused several behavioral alterations, such as uncoordinated swimming movements, hyperactivity, and spasms in the aquatic invertebrates

The results of the present study are in agreement with the few studies mentioned above that investigated the effects of formulated pesticides on biological systems. It is apparent from the present study and the studies mentioned above that the inert ingredients in formulated pesticides have potential adverse toxicological effects on non target aquatic organisms. They enhance the toxicological effects of the active chemical of commercial pesticides. Though no mortalities were observed at the pesticide concentrations used, our results suggest that the well-being of the aquatic invertebrates was greatly affected. Of great concern is the fact that even though, the inert ingredients make up the bulk of a pesticide formulation, there are no laws that bind the pesticide manufacturers to reveal the identity of inert ingredients on product labels, and little information is publicly available about them. [5] also highlighted that manufacturers are not obliged to disclose the inert ingredients in their pesticide formulation even though some of these inert ingredients could be more toxic than the active ingredients of the formulation. It is possible that many non-target aquatic organisms are similarly indirectly exposed and adversely affected by these formulated pesticide products.

CONCLUSION

Inert ingredients in commercial pesticide formulations increased inhibition of esterase activity in *H. duryi* and *L. natalensis* suggesting that some of the ingredients of commercial pesticides may exert adverse effects on aquatic non-target organisms. Toxicological evaluations of pesticides should thus, include toxicological assessments of commercial pesticides both as single entities and in mixtures, to reveal the role played by inert ingredients in the overall toxicity of pesticides. The significant inhibitions of esterase activity in all exposed snails indicated that alterations of esterases in the two snail species have a potential as a biomarkers of exposure to agrochemicals.

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