Bull. Environ. Contam. Toxicol. (2002) 69:586–592

DOI: 10.1007/s00128-002-0101-y

Bulletin of Environmental Contamination and Toxicology

Cadmium-Fenitrothion Interaction in the Spider *Pardosa lugubris* and the Fruit Fly *Drosophila melanogaster*

A. Babczyńska, P. Migula

Department of Animal Physiology and Ecotoxicology, University of Silesia, ul. Bankowa 9, 40-007 Katowice, Poland

Received: 21 October 2001/Accepted: 26 April 2002

Animals inhabiting contaminated environments are, most often, exposed to a wide spectrum of pollutants. Their physiological cost of toxicant tolerance is thus the overall effect of all xenobiotics in their bodies. We usually study in controlled conditions on simple models an exposure to potentially toxic chemicals, which are persistent, e.g. heavy metals, or temporally, like biodegradable pesticides, present in their environments, assessing their effects on animals of various trophic levels. In this study we focused on two widespread chemicals: cadmium, the metal, and fenitrothion, the organophosphate pesticide representing both groups of chemicals. It is generally accepted that cadmium as a biologically unnecessary metal is regarded as highly toxic, disturbing developmental, physiological and genetical processes in both vertebrate and invertebrate species (Hopkin, 1989, Chang, 1996). The metal is transferred along food chains and its concentration in the body may depend on the trophic position of the species. Fenitrothion, [O,O–dimethyl O-(3-methyl-4-nitrophenol) phosphoro-thioate] is one of most effective organophosphate (OP) pesticides which is widely used because of its rapid biodegradation in the environment and a relatively low toxicity to mammals (Worthing, 1983). The OPs and some trace metals are recognized as acetylcholinesterase (AChE) inhibitors. The degree of AChE inhibition has been successfully used as a specific biomarker of exposure to organophosphorous and carbamate pesticides.

The use of insecticides that are toxic to natural enemies, such as spiders, carries the risk of interfering with biological control. On the other hand, spiders are considered to be good indicators of environmental quality since they form species-rich communities in most types of habitats (Foelix, 1996). Therefore in this work an experimental, simple food chain with two invertebrate species, the spider *Pardosa lugubris* (Araneae: Lycosidae) and its prey *Drosophila melanogaster* (Diptera: Drosophilidae) was designed in the laboratory. Both species were exposed to cadmium and fenitrothion using concentrations and exposure times following the pattern of their possible occurrence in nature (Migula, 1997). The aim was to find to what extent toxic activity of fenitrothion might be influenced

Correspondence to: A. Babczyńska

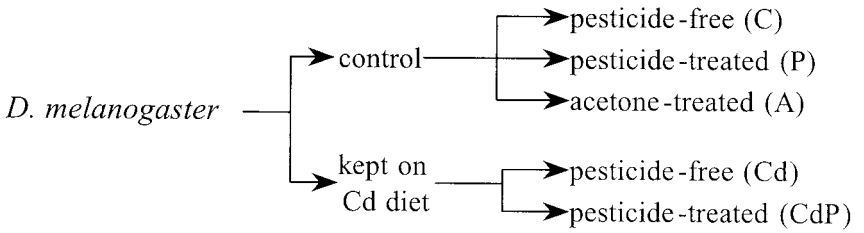

Figure 1. Experimental design for *D. melanogaster*: experimental groups and their symbols used in the text.

by cadmium pretreatment in two species representing the same food chain, assayed on the basis of AChE activity.

MATERIALS AND METHODS

Vestigal fruit fly larvae from the stock culture reared at the University of Silesia were kept for the whole developmental period on artificial diet consisted of sugar, maize cereal, agar, bakery yeast and water, (Graf 1992). For cadmium-treated groups the diet was supplemented with 0.5mM Cd as $CdCl_2$. Adult flies were divided into five experimental groups, two cadmium treated and three Cd-untreated (Fig. 1). Pesticide was used in the concentration of 0.5μM active substance in acetone. Insects were intoxicated through contact with the pesticide solution dissolved on the filter paper in the bottom and by vapours in a 5-cm Petri dish. Juvenile *P. lugubris* collected in an uncontaminated pine forest were kept individually in 150 ml plastic containers and fed *ad libitum* with adult fruit flies until maturation (Foelix, 1996) The spiders were then divided into two main groups of both sexes and subgroups as shown in Fig. 2. Spiders free of excessive cadmium (control) were untreated or treated with fenitrothion and/or acetone. Those fed with cadmium-contaminated fruit flies were treated or untreated with the pesticide (0.5 μM a.i. fenitrothion in acetone solution), applied topically on the upper part of the prosoma, 1μl-drop per female and 0.5 μl-drop per male. One hour after the pesticide or acetone treatment spiders were anaesthetised on ice for biochemical assays. The fruit flies (15-20 per sample) and spiders (1 per sample) were homogenised at 4°C in phosphate buffer, pH 7,4 and centrifuged at 20 000*g* for 10 minutes. AChE activity was determined in supernatants according to Ellman et al. (1961). The reaction was performed at 20°C in 0.35 ml of 0.1M phosphate buffer, pH 8, containing 0.02 ml of 0.01M DTNB (5,5'dithio-(2-nitrobenzoic acid)), 0.02 ml of 0.0075M AChI (acetylthiocholine iodide) as the substrate and 0.01 ml of the supernatant. DTNB and AChI solutions were prepared in phosphate buffers at pHs 7.0 and 8.0, respectively. The reaction was measured photometrically at 410 nm. Enzymatic activity was expressed as μ molAChI/min/mg protein. Protein content was determined according to Bradford (1976). Cadmium was measured in whole animals, dried at 50°C for about 72

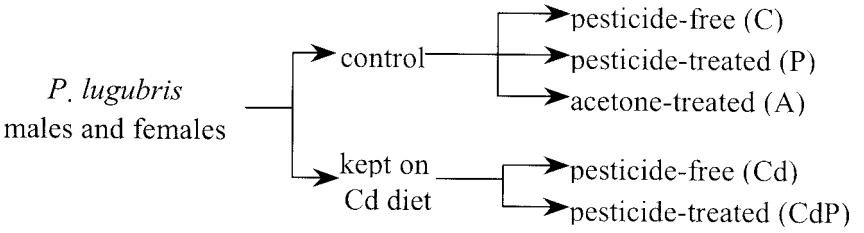

Figure 2. Experimental design for *P. lugubris*: experimental groups and their symbols used in the text.

hours, weighed and digested at 150°C in a mixture of ultapure nitric and perchloric acids in a volumetric ratio of 4:1. Samples were assayed by the AAS method using a Pye Unicam 939 SP-9 with the graphite furnace PU-93090X against standard solutions from Merck at initial concentration of 1g Cd/l water and with SRM 1577b Bovine liver (US Department of Commerce National Institute of Standards and Technology, Gaithersburg MD 20899 and BRC 185 Bovine liver (Institute for Reference Materials and Measurements, Retiesweg B-2440 Geel, Belgium) as reference materials. The LSD test for comparison between experimental groups and the t-test for comparisons between males and females were used from Statistica version 4.5 package for PC.

RESULTS AND DISCUSSION

Exposure to cadmium-contaminated food resulted in 50-fold and 20-fold increase in the metal burden of fruit flies and spiders, respectively, in comparison with the control groups (Tab. 1). It also had a significant effect on AChE activity in females and males of *P. lugubris* (81% and 58% of the control level, respectively, $p<0.001$), but without a significant effect in flies that survived a whole larval period on cadmium-contaminated diet (Tab. 2,3). A possible explanation of this is the possibility of cadmium binding to sulphydryl groups in the enzyme molecule (Devi and Fingerman 1995). These authors showed a similar effect on AChE activity in the crayfish *Procambarus clarkii* exposed for 24 and 48 hours to cadmium, mercury and lead. Lack of similar cadmium-induced effects in fruit flies resulted from differences in developmental patterns. Fruit flies are typical holometabolic insects with no feeding in the pupal stage. Feeding on cadmium-containing diet for the whole developmental period enables the insects to activate protecting mechanisms that reduce its harmful effects. In those that survived the metal exposure, cadmium was effectively bound to metalothioneins (Maroni and Watson, 1985). In flies from the Cd group, where the metal level remained high (Tab. 1) however, the metal did not cause any reduction of AChE activity, compared to the control group. Discussing these inter-specific variances, apart from species specificity we must consider differences in the time of exposition to cadmium between *D. melanogaster* and *P. lugubris*: While 2-weeks time of cadmium intoxication was in spiders only a small part of their ontogenesis, flies

Table 1. Cadmium contents (means ± standard deviation SD) [μg Cd·g dry weight^{-1}] in fruit flies, *D. melanogaster*, and female and male *P. lugubris* fed on the diet not containing (C) or containing (Cd) cadmium.

Group	*Pardosa lugubris*				*D. melanogaster*	
	females		males			
	mean ± SD	n	mean ± SD	n	mean ± SD	n
C	5.9 ± 2.2	4	6.7 ± 2.6	5	6.3 ± 1.3	5
Cd	141.0 ± 25.5	5	116.5 ± 58.3	3	314.7 ±90.2	6

were fed with cadmium-containing diet during the entire developmental period. That time might have been sufficient to activate mechanisms enabling them to prevent harmful effects of the metal to AChE activity. This aspect also has been considered by Schmidt and Ibrahim (1994) who assessed the effects of cadmium on AChE activity in grasshoppers *Aiolopus thalassinus* fed with cadmium-containing food during the whole growth period. In this insect AChE inhibition was relatively low, up to 25%, probably due to protective mechanisms that developed during the period of exposure to the metal. The response to fenitrothion also showed a considerable variation between species. It produced a marked response in *D. melanogaster* (AChE activity reduced to 57% of the value in control flies) but with no significant response in the spiders of either sex. An inhibitory response of AChE activity both in insects and spiders might have been expected. Generally, in many invertebrates AChE inhibition caused by the active forms of organophosphorous compounds is the characteristic deleterious effect of the chemical. It is concentration-and phylogenetic-dependent, as proved for *Chironomus riparius* larvae exposed to methylpirimidophos (Ibrahim et al., 1998), parathion and dichlorphos (Sturm and Hansen, 1999) or *Nereis diversicolor* (Polychaeta) treated with malathion and ethylparathion (Scaps, 1997). The AChE can be adversely affected by the active ingredient in the pesticide formulationand as well by metabolites that can be even more deleterious to organisms (Peakall, 1992). At the cellular level fenitrothion can be oxidised into fenitrooxon, which binds much stronger to the AChE receptors than the parent compound. Escartin and Porte (1996) demonstrated this showing that fenitrooxon is responsible for prolonged AChE inhibition in *Procambarus clarkii* treated with fenitrothion. In our study we showed inhibitory effects of the pesticide. The time lag after the insecticide application was rather short, but cumulative effects of both forms could not be neglected. A substantially weaker reaction to the pesticide of the spider in comparison to insects seems crucial for their survival. In nature the pesticide treatment is more dangerous for them and the toxin may enter their organisms through a direct contact with the sprayed substance and, as well with poisoned insects which might be easier prey to hunt.

The ultimate concern in ecotoxicology is about the character of possible pollutant interactions in animals from various trophic levels. Joint effects of cadmium and fenitrothion on AChE activity once again showed interspecific variation. Both males and females of *P. lugubris* represent a similar type of response: no effects

Table 2. AchE activity [nmol AchI·min^{-1}·mg proetin^{-1}] in *P. lugubris* and *D. melanogaster*: means ±standard deviations SD. Group names – see Fig. 1, 2. [a, b] – different letters (in rows) indicate differences between males and females of *P. lugubris* (Test t, $p<0.05$)

Group	*Pardosa lugubris*				*D. melanogaster*	
	females		males			
	mean ± SD	n	mean ± SD	n	mean ± SD	n
C	11.0 ± 3.4	4	16.0 ± 7.3	5	102.1 ± 36.8	5
Cd	2.0 ± 1.5[a]	5	6.7 ± 0.5[b]	3	91.6 ± 14.5	6
P	6.6 ±3.4	6	8.8 ± 2.9	5	57.7 ± 15.7	5
CdP	4.2 ± 2.1	6	4.4 ± 1.2	3	86.3 ± 11.0	6
A	10.0 ± 5.2	5	6.4 ± 2.3	5	69.8 ± 33.7	4

Table 3. Results of one-way ANOVA ($p > 0,05$) for comparing mean AChE activity in *P. lugubris* and *D. melanogaster* in the experimental groups, with decreasing values. Group names - see Fig. 1, 2. Underlined are homogenous groups.

Species		Experimental groups
P. lugubris	females	C A P CdP Cd
	males	C P Cd A CdP
D. melanogaster		C Cd CdP A P

caused by fenitrothion, and a slight inhibition only in spiders feeding on cadmium treated flies ($p<0.05$). Such differences in AChE response between groups C and CdP were probably due to cadmium-induced alterations on a subcellular level, causing much stronger effects when two stressing factors were present. Worth mentioning is that the pesticide alone did not cause significant alterations of AChE activity in *P. lugubris* (Tabs. 2, 3).In case of *D. melanogaster* the cadmium-fenitrothion interaction is antagonistic: AChE activity depressed in the P., but not in the CdP group. This might be explained by effective binding of cadmium to metalothioneins (Maroni and Watson, 1985) or its storage in type B granules (Hopkin, 1989). This may be beneficial to insects from industrially contaminated areas. Similar effects were also indicated for aquatic animals. Olima et al. (1997) found that AChE inhibition caused by chlorpyriphos was much lower in populations of shrimps (*Parataya australiensis*) exposed to environmental pollutants than in those from unpolluted waters.

In many cases solvents used for pesticide formulation can be also highly toxic. In our studies acetone alone showed a significant, inhibitory, effect only in male spiders (Tabs. 2, 3). Although insecticidal activity of acetone vapours has been demonstrated (Tunç, 1997) further investigation into the mechanism of acetone toxicity is needed.

In conclusion, we have found that the responses of AChE to chemicals acting separately or jointly are strongly variable between fruit flies and spiders. They reflect species specificity and various intoxication periods in relation to the life span of the species. AChE assays in *P. lugubris* and *D. melanogaster* suggest that compensatory mechanisms against potential toxic effects are more efficient in the prey than in the predator. Toxic effects, when manifested at higher trophic levels may eliminate predators from the biota, where they are important in regulating the number of potential herbivorous pests. The experimental use of spiders is difficult for many reasons, thereby the knowledge of their compensatory and adaptive abilities against potential toxins requires further improvements.

REFERENCES

Bradford MM (1976) A rapid method for the quantification of microgram quantities of protein utilising the principle of the protein-dye binding. Anal Biochem 72:248-254

Devi M, Fingerman M (1995) Inhibition of acetylcholinesterase activity in the central nervous system of the red swamp crayfish, *Procambarus clarkii*, by mercury, cadmium and lead. Bull Environm Contam Toxicol 55:746-750

Ellman GL, Courtenay DK, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88-95

Escartín E, Porte C (1996) Bioaccumulation, metabolism and biochemical effects of the organophosphorous pesticide fenithrotion in *Procambarus clarkii*. Environ Toxicol Chem15:915-920

Foelix R. F. (1996) Biology of spiders. Oxford University Press, Georg Thieme Verlag, New York, Oxford

Graf U, Van Schaik N, Wugler FE (1992) *Drosophila* genetics. A practical course. Springer Verlag

Hopkin S.P. (1989) Ecophysiology of metals in terrestrial invertebrates. Elsevier Appl. Science. London New York

Ibrahim H, Kheir R, Helmi S, Lewis J, Crane M (1998) Effects of organophosphorous, carbamate, pyrethroid and organochlorine pesticides and a heavy metal on survival and cholinesterase activity of *Chironomus riparius* Meigen. Bull Environ Contam Toxicol 60:448-455

Maroni G., Watson D. (1985) Uptake and binding of cadmium, copper and zinc by *Drosophila melanogaster* larvae. Insect Biochem 13:55-63

Migula P. (1997) Molecular and physiological biomarkers in insects as the tools in environmental hazard assessment. Acta Phytopathol Entomol Hungary 32:231-243.

Olima C, Pablo F, Lim RP (1997) Comparative tolerance of three populations of the freshwater shrimp (*Parataya australiensis*) to the organophosphate pesticide, chlorpyrifos. Bull Environ Contam Toxicol 59:321-328

Peakall DB (1994) Biomarkers: the way forward in environmental toxicology. Toxicol Ecotoxicol News 1,2:55-60

Scaps P, Demuynck S, Descamps M, Dhainaut A (1997) Effects of organophosphate and carbamate pesticides on acetylcholinesterase and choline acetyltransferase activities of the polychaeta *Nereis diversicolor*. Arch Environ Contam Toxicol 33:203-208

Schmidt GH, Ibrahim NNM (1994) Heavy metal content (Hg^{2+}, Cd^{2+}, Pb^{2+}) in various body parts: its impact on cholinesterase activity and binding glycoproteins in the grasshopper *Aiolopus thalassinus* adults. Ecotoxicol Environ Safety 29:148-164

Sturm A, Hansen P-D (1999) Altered cholinesterase and monooxygenase levels in *Daphnia magna* and *Chironomus riparius* exposed to environmental pollutants. Ecotoxicol Environ Safety 42:9-15

Thompson HM (1999) Esterases as biomarkers of exposure to organophosphates and carbamates. Ecotoxicology 8:369-384

Tunç I., Erler F., Dagli F., Çalis Ö. (1997) Insecticidal activity of acetone vapours. J Stored Prod Res 33:81-185

Worthing CR (Ed) (1983) The pesticide manual: A world compendium. The British Crop Protection Council, Croydon, England