Bull. Environ. Contam. Toxicol. (2004) 72:1247–1252

DOI: 10.1007/s00128-004-0377-1

Bulletin of Environmental Contamination and Toxicology

Effects of Cimetidine and Phenobarbital on Methyl-Parathion Metabolism in *Hyalella azteca*

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Received: 11 June 2003/Accepted: 10 March 2004

It has been suggested that lipoperoxidation is involved in organophosphorus pesticide metabolism (Yarsan et al. 1999). It is also known that cell membrane damage occurs as a consequence of this process (Khrer 1993). On the other hand, there are certain vitamins and enzymes that can reduce lipoperoxidation. Glutathione-S-transferase, glutathione peroxidase, glutathione reductase, and gamma-glutamyltranspeptidase are known to need glutathione and used by most cells to inhibit this effect (Repetto 1997). Gamma-glutamyltranspeptidase (GGT)is a membrane-bound enzyme for which enrichment in cells has been shown to result in an increased resistance against prooxidant injury (Paolicchi et al. 1997).

Methyl-parathion conversion to paroxon takes place in cells by microsomal monooxygenases (FMO) (Butler and Murray 1993). Methyl-parathion biotransformation in aquatic organisms can be significantly affected by exposure to environmental contaminants such as triphenyltin and methidathion which are known to inhibit FMOs, while polycyclic aromatic hydrocarbons and polychlorinated biphenyls tend to stimulate this system (Whyte et al. 2000). FMO can be affected also by other agents such as cimetidine (Chang et al. 1992) and phenobarbital (Hodgson 1994) since they can change the most toxic metabolite level (Stegeman and Hahn 1994). This study aimed to evaluate methyl-parathion toxicity by estimating GGT activity in *Hyalella azteca* and lipoperoxidation using cimetidine and phenobarbital.

MATERIALS AND METHODS.

H azteca organisms were collected from Xochimilco Lake México. All organisms were kept at 25°C with a photoperiod of 16:8 (cool-white fluorecent light). The physicochemical properties of the reconstituted water (6.0 g $CaSO_4.2H_2O$, 6.0 g $MgSO_4$, 9.6 g $NaHCO_3$, and 0.4 g KCl in 100 mL of water deionized) were as follows: hardness (as $CaCO_3$) = 80 to 100 mg/L; alkalinity (as $CaCO_3$) = 60 to 70 mg/L; pH 7.4 to 7.8, and dissolved oxygen 5.5 to 6.5 mg/L.

Cultures of 120 adult organisms were reared in glass quart jars containing six 5 X

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10 cm pieces of pre-soaked cotton gauze (3 mm mesh cheesecloth). These cultures were aerated, and fed with *Chlorella sp* (1 X 10^6 cells/mL) daily. Cultures were sieved every 7 d to remove the young for bioassays and to start new cultures. During the sieving process, 25 to 30 % of the culture water was replaced with fresh culture water, and gauze was added as needed to keep a relatively constant volume (Kubitz and Giesy 1996).

The technical methyl-parathion (95.3 % pure) (MP) was donated by TeKchem, Cimetidine (54 % pure) (Ci) is made by Smith Kline Beechman (Mexico), Phenobarbital (PhB) was purchased from Sigma.

Amphipod cultures were kept at a constant temperature, 25°C, under a 16 hr light: 8 hr dark regimen. *H. azteca* acute bioassays were performed with 3 to 10 d old juveniles. 20 animals were used for every concentration or combination of concentrations used. They were put in 250 mL glass containers, containing 100 mL of test solution with three replicates/ treatment. The acute toxicities of MP (0.01, 0.1, 1.0, 10.0, 100 µg/L), Ci (0.01, 0.1, 1.0, 10.0, 100 mg/L), MP+Ci (0.001 +100, 0.01+100, 0.1+100, 1.0+100, 10 + 100 mg/L), PhB (0.01, 0.1, 1.0, 10.0, 100 mg/L), MP+ PhB (0.01 µg/L+ 100 mg/L, 0.1 µg/L+ 100 mg/L, 1.0 µg/L+ 100 mg/L, 10 µg/L + 100 mg/L, 100 µg/L + 100 mg/L,) were estimated in *H. azteca*, for a 48 hr period. A 0.6 % acetone in 0.4% ethanol solution and 0.4 % ethanol solution were used to dissolve the pesticide and Ci respectively. A control test was conducted in reconstituted water without pesticide, but with ethanol and acetone. There were 5 replicates of all tests.

The behavior and survival were observed for all agents and compared with those of controls. The absence of response to a gentle mechanical stimulus was the criterion for death. The median effective concentration after 48 hr (48-hr EC_{50}) for immobilization was calculated by computerized log-probit analysis.

Six 30-organism (30 d old) groups were exposed to sublethal concentrations of the following agents for 10 d: 1) reconstituted water + acetone (0.6%) in ethanol 0.4%, 2) PhB =100 mg/L, 3) Ci=100 mg/L, 4) MP=40.4 µg/L, 5) MP + PhB= 0.0195 µg/L +100 mg/L, 6) MP + Ci=40.4 µg/L +100 mg/L. Five replicates were maintained for each of the concentrations tested. They were put in 250 mL vessels containing 100 ml of test solution. Every other day all animals were fed with *Chlorella sp* (1 X 10^6 cells/mL). After the exposure period, organisms were homogenized at 4°C with a 0.15 M sodium chloride solution, then centrifuged at 14,000 g for 15 min, then the resulting supernatant was centrifuged again at 3500 g for 5 min. In this latter supernatant the GGT activity was determined by the Huseby and Strome method (1974), while lipoperoxidation and total protein content were estimated by the Buege and Aust (1978) and Bradford (1976) methods respectively.

Data were statistically analyzed using one way ANOVA and mean differences of each group were compared using the Tukey test. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The value of the 48 hr-EC_{50} for MP in the amphipod was very similar to that obtained in *Daphnia magna* (0.14 µg/L) (EPA 1998). However, the MP+Ci EC_{50} value was greater than that of MP (Table 1).

Table 1. Acute toxicity of methyl-parathion, cimetidine and phenobarbital on *Hyalella azteca.*

	48 hr-EC_{50} (µg/L)
methyl-parathion. 95% confidence limit	0.172 (0.0843- 0.3512)
cimetidine.	*
phenobarbital.	*
methyl-parathion plus cimetidine. 95% confidence limit	40.4 (19.8- 82.4)
methyl-parathion plus phenobarbital. 95% confidence limit	1.0 (0.4899- 2.0413)

(*) mortality was not observed until the concentration of 100 ppm

Mourelle et al. (1986) report that cimetidine inhibits the FMO system which metabolizes the pesticide. On the other hand, the MP+PhB EC_{50} value obtained was 5.8 times greater than that of MP. A related study points out that phenobarbital stimulates other biotransformation routes (Fischer and Varga 1985). Glutathione S-transferase is induced by phenobarbital, this enzyme can remove methyl groups from the organophosphorus insecticides that contain the CH_3 -O-P group (Hassall 1990). Joshi and Thornburg (1986) found similar effects with these xenobiotics in rodents.

Sublethal toxicity studies using MP+Ci in the amphipod demonstrated a greater production of malondialdehyde than the of the control group (5000 times) or the MP group (294 times) (Figure 1) ($p<0.05$). This result shows a different metabolite than paroxon or *p*-nitrophenol is giving rise to the lipoporoxidation process, since cimetidine inhibits pesticide metabolism (Mourelle et al. 1986). This metabolite might be an unstable phosphooxathiiran intermediate (Straus et al. 2000). This compound is a highly reactive electrophile which would be expected to bind readily to nucleophiles near the site of its release (Neal and Halpert 1982). This latter hypothesis is supported by the EC_{50} value obtained when organisms were exposed to MP+Ci. It must be emphasized that this value was 234 times greater than that of MP. Lipoperoxidation activation by the unstable metabolite is also supported by the fact that those organisms exposed to MP+PhB showed a lipoperoxidation index approximately equal to the sum of the two lipoperoxidation levels observed for each isolated xenobiotic (MP= 0.00129 or PhB= 0.0027µmol MAD/ mg protein).

The available data on experimental animals and in vitro studies indicate that

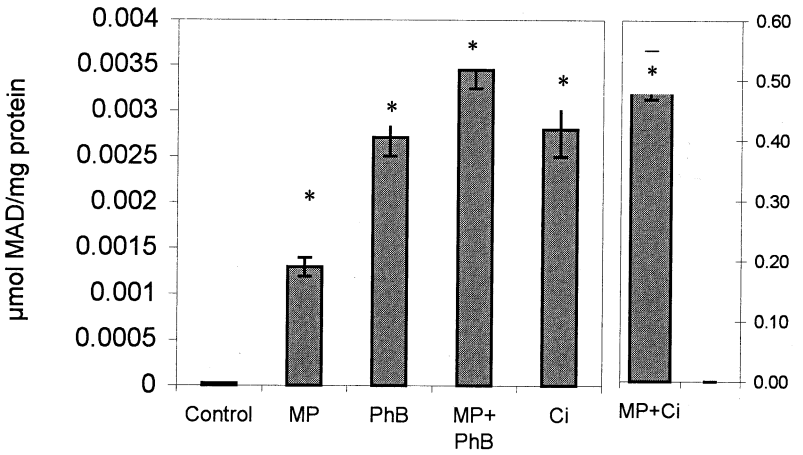

Figure 1. *Hyalella azteca* malondialdehyde levels in treated groups with: methyl-parathion (MP); phenobarbital (PhB); MP + PhB; Cimetidine (Ci); MP + Ci. ; * indicates a significant difference from control ($P<0.05$).

pesticides alter enzymes associated with antioxidant defense mechanisms (El-Sharkawy et al. 1994). In this work it was found that the amphipod GGT was increased at least 177 times with MP+ Ci compared to that obtained for each isolated xenobiotic (cimetidine or methyl-parathion) and it increased 10,500 times with respect to control (Figure 2) ($p<0.05$).

This latter effect may be due to an adaptive process to accumulated metabolite free radicals from MP. GGT participates in glutathione (GSH) synthesis, which is used to eliminate electrophilic compounds (Banerjee et al. 1999). Paolicchi et al (1997) suggest that the role of this enzyme rolle is to increase cell antioxidant mechanisms.

GGT activity decreased 82.3 % in the group exposed to MP plus PhB compared to that of the group exposed to MP (Figure 2) ($p<0.05$). It is well known that GGT requires the participation of GSH as well as a wide range of amino acids as substrates (Meister 1981). Since amino acid content in the metabolic pool is expected to decrease as a result of pesticide conjugation (Hutson 1981), it is not surprising, therefore, to find a decrease in the activity of GGT, which utilizes amino acids. It is also important to mention that when the insecticide biotransformation is stimulated by PhB there will be an increase in hydrosoluble metabolites.

The result from the present study indicate that paroxon or p-nitrophenol my not be responsible for the lipoperoxidation process.

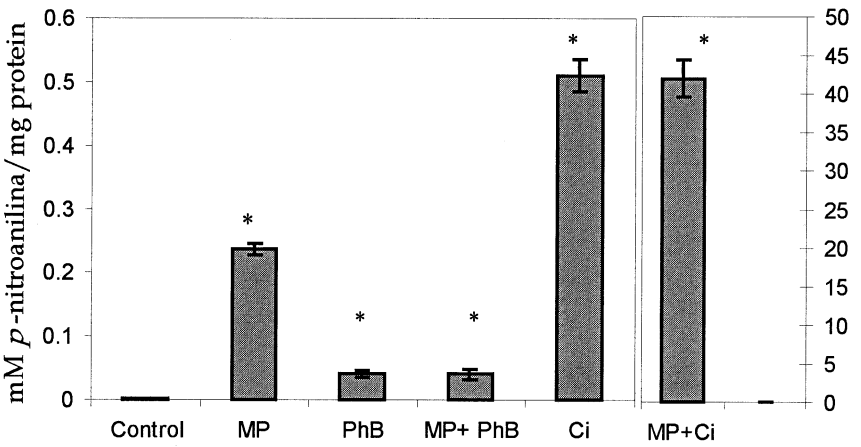

Figure 2. Gamma glutamyltranspeptidase activity in homogenized *Hyalella azteca* exposed to methyl-parathion (MP); phenobarbital (PhB); MP + PhB; Cimetidine (Ci); MP + Ci; * indicates a significant difference from control ($P<0.05$).

REFERENCES

Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK (1999) Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett 107:33-47

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

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Meth Enzymol 52: 302-310

Butler AM, Murray M (1993) Inhibition and inactivation of constitutive cytochromes P450 in rat liver by parathion. Mol Pharmacol 43: 902-908

Chang T, Levine M, Bandiera SM, Bellward GD (1992) Selective inhibition of rat hepatic microsomal cytochrome P-450. I. Effect of the *in vivo* administration of cimetidine. J Pharmacol Exp Ther 260:1441-1449

El-Sharkawy AM, Abdel-Rahman SZ, Hassan AA, Gabr MH, El-Zoghby SM El-Sewedy SM (1994) Biochemical effects of some insecticides on the metabolic enzymes regulating glutathione metabolism. Bull Environ Contam Toxicol 52:505-510

EPA (Environmental Protection Agency) (1998) Methyl-parathion Environmental fate and effects. Preliminary risk assessment. Pesticides and Toxic Substances, Washington DC, EPA 2460

Fischer E, Varga F (1985) Substrate and phenobarbital induction of the biliary excretion of exogenous organic anions in rats. Arch Toxicol Suppl 8:345-347

Hassall K. 1990. The biochemestry and uses of pesticides. Macmillan Press LTD, London, pp 74-75

Hodgson E (1994) Chemical and environmental factors affecting metabolism of

xenobiotics. In: Hodgson E, Levi PE (eds) Introduction to Biochemical Toxicology. Appleton & Lange, Norwalk, Connecticut, p 164

Huseby NE, Stromme JH (1974) Practical points regarding routine determination of gamma-glutamyl transferase (gamma-GT) in serum with a kinetic method at 37 degrees C. Scand J Clin Lab Invest 34: 357-363

Hutson DH (1981) The metabolism of insecticides in man. In: Wiely J, Hotson DH, Roberts TR (eds) Progress in Pesticide Biochemistry, Vol 1. John Wiley & Sons, England, p 85

Joshi UM, Thornburg JE (1986) Interactions between cimetidine, methylparathion, and parathion. J Toxicol Environ Health 19:337-344

Khrer JP (1993) Free radical as mediator of tissue injury and disease. Crit Rev Toxicol 23: 21-48

Kubitz JA, Giesy JP (1996) Changes in overlaying water quality and sediment toxicity to *Hyalella azteca* during a 10-day sediment bioassay: Static vs. renewal regimens. In: Munawar M, Dave G (eds) Development and Progress in Sediment Quality Assessment: Rationale, Challenges, Techniques and Strategies, SPB Academic Publishing, Amsterdam, The Netherlands, p 101

Meister A (1981) On the cycles of glutathione metabolism and transport. Curr Top Cell Regul 18:21-58

Mourelle M, Giron E, Amezcua JL, Martínez-Tabche L (1986) Cimetidine enhances and phenobarbital decreases parathion toxicity. J Appl Toxicol 6:401-404

Neal RA, Halpert J (1982) Toxicology of thiono-sulfur compounds. Ann Rev Pharmacol Toxicol 22:321-339

Paolicchi A, Tongiani R, Tonarelli P, Comporti M, Pompella A (1997) Gama-glutamil transpeptidase-dependent lipid peroxidation in isolated hepatocytes and HepG2 hepatoma cells. Free Rad Biol Med 22:853-860

Repetto M (1997) Toxicología Fundamental. Díaz de Santos, España

Stegeman JJ, Hahn ME (1994) Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions and regulation of cytochrome 450 in aquatic species. In: Malins DC, Ostrander GK (eds) Aquatic Toxicology, Molecular, Biochemical and Cellular Perspectives, Lewis Publishers, Boca Raton, FL, p 87-206

Straus DL, Schlenk D, Chambers JE (2000) Hepatic microsomal desulfuration and dearylation of clorpyrifos and parathion in fingerling channel catfish: lack of effect from Aroclor 1254. Aquat Toxicol 50: 141-149

Whyte JJ, Jung RE, Schimitt CJ, Tillitt DE (2000) Ethoxyresorufin-Odeethylase (EROD) activity in fish as a biomarker of chemical exposure. CRC Crit Rev Toxicol 30:347-570

Yarsan E, Tanyuksel M, Celik A, Aydin A (1999) Effects of aldicarb and malathion on lipid peroxidation. Bull Environ Contam Toxicol 63: 575-581