Bull Environ Contam Toxicol (2009) 83:334–336
DOI 10.1007/s00128-009-9741-5

Removal of Methyl Parathion in Water, by *Dugesia dorotocephala*

A. Amaya-Chávez · E. López-López · M. Galar-Martínez · L. M. Gómez-Oliván · M. M. García-Fabila

Received: 29 September 2008 / Accepted: 23 April 2009 / Published online: 8 May 2009

Abstract The aim of this study was to determine the efficiency of *Dugesia dorotocephala* on Methyl parathion removal. An initial concentration of 1.25 μg mL^{-1} of MeP was used to evaluate the removal capacity of planarian. A first-order removal kinetics was obtained with a disappearance rate constant (k_r) of 0.49 $days^{-1}$ and 69% efficiency on contaminant removal. This is significantly different ($p < 0.5$) from the degradation occurring in control systems, leading us to conclude that *D. dorotocephala* effectively removes MeP from contaminated water.

Keywords *Dugesia dorotocephala* · Methyl parathion removal · Acethylcholinesterase

Widespread use of organophosphorus (OP) insecticides, such as methyl parathion (MeP), has significantly increased their load in the environment, contaminating aquatic systems directly or through soil permeation, agricultural drainage, or wastewater generated during their production (Biziuk et al. 1996). The amount of pesticides in surface water and sediment depends primarily on the proximity of the latter two to food-producing areas in a given region. Thus, rivers and lakes surrounded by agricultural areas show significant pesticide levels.

In the United States, MeP has been found in surface water at levels above 0.46 μg L^{-1} and in underground water near point-contamination sources at 0.01 μg L^{-1} (ATSDR 2001). Recent studies in Mexico have detected OP pesticides in the water of different reservoirs. Thus, Favari et al. (2002) report presence of MeP in samples of Ignacio Ramírez reservoir water (0.21×10^{-3}–0.35×10^{-3} mg L^{-1}), phytoplankton (0.17×10^{-3}–2.17×10^{-3} mg Kg^{-1}) and fish muscle (0.4×10^{-3}–0.97×10^{-3} mg Kg^{-1}). Similarly, Agapito de la Cruz (2004) found concentrations of 0.004–0.39 mg L^{-1} in water and 3.1–60.8 mg kg^{-1} in sediment from the Lechuguilla-Ohuira-Navachiste Basin in Sinaloa.

Microorganisms and plants have been traditionally used to remove xenobiotics. Recently however, benthic organisms, which help eliminate contaminants due to their bioconcentration capacity, have also been used. De la Vega et al. (1997) showed that *Cambarellus montezumae, Mollusca-Gastrododa Physidae* and *Girardinichthys multiradiatus* are able to bioconcentrate OP pesticides (MeP). Martínez-Tabche et al. (2002) showed fast bioaccumulation of malathion in the snail *Stagnicola* sp., as did also García (1999) in the worm *Limnodrillus hoffmeisteri*.

Planarian worms are benthic organisms. Some 100 species are known to exist. They are an important component of aquatic ecosystems due to their biotic interactions with other benthic communities, their contribution to the diet of other organisms and their involvement in photosynthesis (Newmark and Alvarado 2002). Kouyoumjian

A. Amaya-Chávez (✉) · L. M. Gómez-Oliván · M. M. García-Fabila
Departamento de Farmacia de la Facultad de Química, UAEMex., Paseo Tollocan Esq., Paseo Colón, Col., Residencial Colon, CP 50100 Toluca, Estado de México, Mexico
e-mail: aamayac@uaemex.mx

E. López-López
Departamento de Zoología, Laboratorio de Ictiología y Limnología, Escuela Nacional de Ciencias Biológica, IPN, Prolongación de Plan de Ayala Esq. con Carpio, Delegación, Miguel Hidalgo, CP 11340 Mexico, DF, Mexico

M. Galar-Martínez
Departamento de Farmacia, Escuela Nacional de Ciencias Biológica, IPN, Prolongación de Plan de Ayala Esq. con Carpio, Delegación, Miguel Hidalgo, CP 11340 Mexico, DF, Mexico

and Villeneuve (1979) showed that planarians are able to slowly absorb sublethal concentrations of diclorodifenil-tricloroetano (DDT) in water, also finding its metabolites (diclorodifenildicloroetileno (DDE) and diclor-odifenildicloroetano (DDD).

The aim of this study was to determine the efficiency of *Dugesia dorotocephala* on Methyl parathion removal.

Materials and Methods

The planarian species used in this study was *Dugesia dorotocephala*, which were cultured during 3 months prior the study in 20 L glass containers placed in a shaded area, with tap water (pH 6.8–7.2), at room temperature (18–22°C), natural light/dark periods and constant aeration. They were fed chicken liver weekly to satiety as suggested by Kostelecky et al. (1989).

In order to establish the MeP concentration at which planarian was tolerant, the MeP mean lethal concentration (LC_{50}), as well as the acethylcholinesterase activity (AChE), were determined. The latter due that the main action mechanism of OP insecticides, is the inhibition of this esterase (Buttarelli et al. 2000).

The acute toxicity assay was conducted using six 300 mL glass containers filled with 200 mL of an aqueous solution of MeP at different concentrations of the active ingredient (control, 1, 3, 5, 7 and 9 mg L^{-1}), and 10 planarian maintained under the same conditions as for culture. Mortality rates were determined at 24, 48, 72 and 96 h. The experiment was performed in triplicate. The LC_{50} was estimated by the Probit method.

The AChE activity assay was conducted using six lots of ten planarian in containers similar to those used in the LC_{50} assay and maintained under similar conditions. A 1.25 mg L^{-1} concentration, equal to 1/5 of the LC_{50} value determined earlier in the acute assay, was added to five of the test systems and exposed during 2, 4, 6, 8 and 10 days. The sixth was used as control. At the end of each exposure period, 0.5 g of surviving organisms were taken, suspended in 20 mL Tris HCl pH 7.5 and homogenized and centrifuged at 12,000 rpm and 4°C by 10 min. The supernatant was used to determine AChE activity (Hestrin 1949). Percentage of AChE inhibition was estimated by taking the basal enzyme activity of test organisms as 100%.

The MeP removal assay was performed using six test systems, added with1.25 mg L^{-1} of MeP (amount at which no significant differences in AChE activity were found with respect to controls). Ten planarian were placed in five of the test systems but not in the sixth one, which was used as control. The initial and residual insecticide levels in water were measured by gas chromatography every 48 h until a minimum quantifiable concentration was obtained in both; control systems (without planarian) and test systems (Perkin–Elmer Autosistem XL; detector: FID, 0.25 mm ID, 30 m long, 0.25 df dimethyl polysiloxane capillary column, injector temperature 280°C, detector temperature 280°C; initial oven temperature 70°C for 1 min, then increased at 12°C per min to 250°C, held for 2 min and ramped again at 15°C per min to 280°C, concentration range 8–100 mg L^{-1}; detector: ECD, detector temperature 300°C, concentration range 1–12 mg L^{-1}). AChE activity was quantified after 0, 2, 4, 6, 8 and 10 days in order to verify the health of the test organisms. The experiment was performed in triplicate. Determination was made of the equation describing the kinetics process and the corresponding parameters.

Biochemical and MeP removal efficiency data were evaluated by analysis of variance (ANOVA) using SIGMA STAT v.3 software.

Results and Discussion

The LC_{50} value obtained in the acute toxicity assay was 5.58 mg L^{-1} at 96 h, with a confidence interval of 4.20–6.97. The χ^2 test for goodness of fit was not significant at $p < 0.05$. This is consistent with the value found by Villar and Schaeffer (1993), who report an LC_{50} of 4.2 mg L^{-1} in the same species after 7 days of exposure at 19°C, as well as with the LC_{50} of 4.1 mg L^{-1} observed in the species *D. trigrina* after 96 h of exposure at 19°C (Villar et al. 1993).

Since the AChE inhibition is the main action mechanism of MeP, it was decided to use this enzyme activity as a biomarker in order to determine the pesticide concentration and exposure time tolerated by the test organism and be able to evaluate its removal efficiency. A 1.25 mg L^{-1} concentration, equal to 1/5 of the LC_{50} value determined earlier in the acute assay was used, founding AChE activity was constant (Fig. 1) and equal to control values ($p \leq 0.05$), and therefore the planarian health.

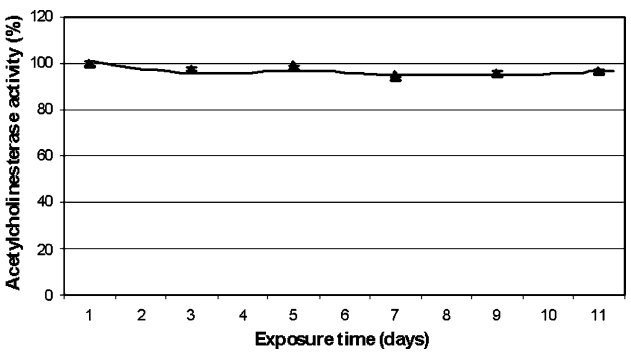

Fig. 1 Acetylcholinesterase (AChE) activity in planarian exposed to 1.25 mg L^{-1} of MeP. Results show the mean of three determinations and corresponding SD at a 0.05 level of significance

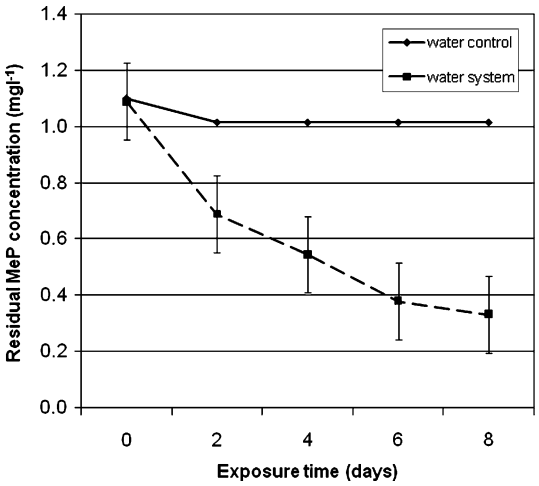

Fig. 2 Removal kinetics of 1.25 mg L^{-1} of MeP by planarian in water. Results show the mean of three determinations and corresponding SD at a 0.05 level of significance

The kinetics of MeP removal by planarian in water (Fig. 2) adheres to a first-order process, with a disappearance rate constant (k_r) of 0.493 $days^{-1}$ and a removal half-life ($t_{1/2}$) of 4.64 $days^{-1}$. The degradation half-life of MeP in river water in the absence of any treatment and under environmental conditions similar to those used in this study (22°C temperature and pH 7.3) is reported to be 23 days (Lartiges and Garrigues 1995). Thus, it is evident that our model accelerates five-fold the degradation of this compound. Also, comparison of results obtained in control systems without planarian and test systems with planarian (Fig. 2) using one-way ANOVA found a significant difference between the two ($p < 0.05$). Planarian showed 68.8% MeP removal efficiency at 8 days, while pesticide degradation in control systems during the same period was 7.27%. Removal was accelerated five-fold in test systems with planarian. This species may therefore be a good candidate for use as a bioremedial organism in MeP contaminated surface water.

Acknowledgments The authors thank Ruth Georgina Salinas Gómez (Laboratorio de Instrumental, Facultad de Química, UAEMex) for GC analytical support and Leonor Miranda Monroy (Laboratorio de Ciencias Ambientales, Facultad de Química, UAEMex) for assistance in organisms culture.

References

Agapito de la Cruz MR (2004) Patrón de uso de agroquímicos y concentración de plaguicidas organofosforados en agua y sedimento de la cuenca Lechuguilla-Ohuira-Navachiste, Sinaloa, México. B. S. Tesis, Escuela de Biología, Universidad Autónoma de Sinaloa

ATSDR, Agency for Toxic Substances and Disease Register (2001) Toxicological profile for methyl parathion, update. Department of Health and Human Services, Public Health Service, Atlanta GA

Biziuk M, Przyjazny A, Czerwinski J (1996) Occurrence and determination of pesticides in natural and treated waters. J Chromatogr 754:103–123. doi:10.1016/S0021-9673(96)00297-X

Buttarelli RF, Pontieri EF, Margotta V, Palladini G (2000) Acetylcholine/dopamine interaction in planaria. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 125:225–231. doi:10.1016/S0742-8413(99)00111-5

De la Vega SM, Martínez TL, Macías GC (1997) Bioaccumulation of methyl parathion and its toxicology in several species of the freshwater community in Ignacio Ramirez Dam in Mexico. Ecotoxicol Environ Saf 38:53–62. doi:10.1006/eesa.1997.1551

Favari L, López E, Martínez-Tabche L, Díaz-Pardo E (2002) Effect of insecticides on plankton and fish of Ignacio Ramírez Reservoir (México). A biochemical and biomagnification study. Ecotoxicol Environ Saf 51:177–186. doi:10.1006/eesa.2002.2142

García LR (1999) Relación de la captación de malatión en sedimentos artificiales y su efecto tóxico sobre *Limnodrilus hoffmeisteri*. Tesis de maestría en Toxicología, ENCB. IPN

Hestrin S (1949) The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. J Biochem 3:249–259

Kostelecky J, Elliott B, Schaeffer DJ (1989) Physiology of sexual-only *Dugesia dorotocephala:* effects of diet and population density on adult weight and cocoon production. Ecotoxicol Environ Saf 18:286–295. doi:10.1016/0147-6513(89)90022-5

Kouyoumjian HH, Villeneuve JP (1979) Further studies on the toxicity of DDT to planaria. Bull Environ Contam Toxicol 22:109–112. doi:10.1007/BF02026916

Lartiges SB, Garrigues PP (1995) Degradation kinetics of organophosphorus and organonitrogen pesticides in different waters under various environmental conditions. Environ Sci Technol 29:1246–1254. doi:10.1021/es00005a016

Martínez-Tabche L, Galar-Martínez M, Olvera HE, Chehue RA, López-López E, Gómez-Oliván L, Terrón SO (2002) Toxic effect and bioavailability of malathion spiked in natural sediments from the Ignacio Ramírez dam on the snail *Stagnicola* sp. Ecotoxicol Environ Saf 52:232–237. doi:10.1006/eesa.2002.2162

Newmark PA, Alvarado SA (2002) Not your father's planarian: a classic model enters the era of functional genomics. Nature Rev Genet 3:210–219. doi:10.1038/nrg759

Villar D, Schaeffer J (1993) Morphogenetic action of neurotransmitters on regenerating planarians – a review. Biomed Environ Sci 6:327–347

Villar D, Mei-Hui L, Schaeffer J (1993) Toxicity of organophosphorus pesticides to *Dugesia dorotocephala*. Bull Environ Contam Toxicol 51:80–87. doi:10.1007/BF00201004