Bull. Environ. Contam. Toxicol. (2003) 70:146–152

DOI: 10.1007/s00128-002-0168-5

Bulletin of Environmental Contamination and Toxicology

Toxic Effects of Dimethoate and Carbaryl Pesticides on Protein Metabolism of the Freshwater Snail *Lymnaea acuminata*

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Received: 25 January 2002/Accepted: 14 August 2002

Dimethoate (organophosphate) and carbaryl (carbamate) pesticides are widely used in agricultural fields to control agricultural and aquatic pests and aquatic weeds. These pesticides reach in water bodies by agricultural run off or irrigation (Li 1975), which cause adverse effects on the aquatic fauna. Both pesticides inhibit the activity of acetylcholinesterase (AChE) and cause death, (Coppage and Matthews 1974; Bocquéné and Galgani 1991). Because of their electrophilic nature these pesticides also act on other enzymes necessary for oxidative metabolism. Hypoxia occurs in aquatic organisms due to the presence of these pesticides in water bodies. Carbohydrates are the chief and immediate source of energy while proteins are the source of energy during chronic periods of stress (Umminger 1977).

The freshwater snail *Lymnaea acuminata* is a cosmopolitan aquatic organism and important primary consumer in many freshwater bodies (Burris et al. 1990). This snail is important in the detritus food chain of aquatic ecosystem. Hypoxia resulting from these pesticides altered the carbohydrate metabolism of the snails lowering energy production. Proteins are the alternative source of energy during stress. So, the aim of this investigation is to measure the effects of different sub-lethal exposures of dimethoate and carbaryl pesticides on protein metabolism of the freshwater snail *Lymnaea acuminata*.

MATERIALS AND METHODS

The adult freshwater snails *Lymnaea acuminata*, of almost uniform size range, (36.4±1.8 mm shell height and 20.2±1.2 mm shell width) were collected from non-contaminated waters of Gorakhpur district of Uttar Pradesh and kept in glass aquaria containing 30 L dechlorinated tap water for at least 96h to acclimatize them to laboratory conditions. Water was changed every day. Dead snails were removed as soon as possible to avoid water fouling. The snails were fed daily on washed and dried *Nymphaea* leaves during the whole acclimatization period. Dimethoate (O, O- dimethyl S- (N-methylcarbamoylmethyl) phosphorodithioate) and carbaryl (1- naphthyl – N – methylcarbamate) were used as the organophosphate and carbamate pesticides, respectively. The LC_{50} values of dimethoate are 19.7 mg/L and 10.8 mg/L for 24h and 96h, respectively, while

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LC_{50} values of carbaryl are 20.1 mg/L and 14.2 mg/L for 24h and 96h, respectively for the freshwater snail *Lymnaea acuminata* (Srivastava and Singh 2001). Sub-lethal doses (i.e. 3.0 mg/L, 6.0 mg/L, 9.0 mg/L and 12.0 mg/L) of both the pesticides were used for biochemical experiments.

The desired amount of pesticides was mixed in glass aquaria containing 5 L dechlorinated tap water. Twenty snails were placed in the each aquarium. In control groups the water was pesticide free. The water temperature was kept at 23±1°C during the testing. No food was given to the snails during the course of experiment. Snails were treated for 24h and 96h and after completion of treatment they were dissected and hepatopancreas (digestive organ) and ovotestis (reproductive organ) tissues were removed for biochemical analysis.

Total protein was measured according to Lowry et al. (1951). Homogenate (50 mg/mL, w/v) was prepared in 10% TCA. Standard curves were prepared with different concentrations of bovine serum albumin. Value expressed as μg protein/mg of tissue. Free amino acid level was measured according to Spies (1957). Homogenate (50 mg/mL, w/v) was prepared in 96% ethanol. Standard curves using the same procedure were drawn with known amounts of glycine. Value expressed as μg/mg of tissue. Nucleic acids (DNA & RNA) were estimated according to Schneider (1957) using diphenylamine and orcinol reagents, respectively. Homogenate (50 mg/mL, w/v) was prepared in 5% TCA at 90°C. Both DNA and RNA have been expressed as μg/mg tissue. Protease activity was measured according to Moore and Stein (1954). Homogenate (50 mg/mL, w/v) was prepared in cold distilled water. The enzyme activity was expressed in μ moles of tyrosine equivalent/mg protein/h.

Each assay was replicated six times, values are expressed as mean ±SE of six replicates and Student's 't' test was applied to locate significant ($P<0.05$) differences between treated and control groups

RESULTS AND DISCUSSION

Data of biochemical analysis in the hepatopancreas and ovotestis tissue of the snail after exposure to different sub-lethal doses are given in Tables 1, 2 and 3. Dimethoate and carbaryl pesticides significantly alter the levels of total protein, free amino acid and nucleic acids (DNA & RNA) and the activity of protease in hepatopancreas and ovotestis tissues (Tables 1-3).

The data clearly indicate that levels of total protein and nucleic acids were reduced upon exposure in both tissues, while free amino acid level was increased. Total protein level was maximally reduced in hepatopancreas after exposure to 24h and 96h against dimethoate and carbaryl. DNA level was maximally reduced in ovotestis tissue after exposure to 24h and 96h against dimethoate and carbaryl. RNA level was maximally reduced in ovotestis tissue after 24h and 96h exposure to dimethoate and carbaryl. Free amino acids level was maximally increased in the

Table 1. Total protein (TP) (μ g/mg), free amino acid (FAA) (μ g/mg) and nucleic acids (DNA & RNA) (μ g/mg) in hepatopancreas (HP) and ovotestis (OT) tissues of freshwater snail *Lymnaea acuminata* after dimethoate exposure.

	Tissue	Exposure period	Control	Dimethoate dose			
				3.0 mg/L	6.0 mg/L	9.0 mg/L	12.0 mg/L
TP	HP	24h	55.3±5.0 (100)	52.0±4.7 (94)	49.3±4.2* (89)	44.8±4.0* (81)	38.7±3.9* (70)
		96h	54.7±4.8 (100)	50.9±4.5 (93)	47.1±4.1* (86)	43.2±4.0* (79)	36.1±3.8* (66)
	OT	24h	70.6±6.5 (100)	67.8±6.3 (96)	64.3±5.9 (91)	60.0±5.7* (85)	55.8±5.7* (79)
		96h	68.56.1 (100)	64.4±6.1 (94)	60.9±6.0* (89)	56.1±5.5* (82)	52.0±5.0* (76)
FAA	HP	24h	17.9±2.0(100)	19.0±2.1 (106)	20.8±2.1* (116)	22.8±2.2* (127)	25.1±2.1* (140)
		96h	17.72.1 (100)	19.3±2.1 (109)	21.2±2.1* (120)	22.9±2.2* (130)	25.8±2.1* (146)
	OT	24h	16.41.7 (100)	17.7±1.8 (108)	19.5±2.1* (119)	21.4±2.1* (131)	23.5±2.2* (144)
		96h	16.31.6 (100)	17.9±1.7 (110)	19.8±2.0* (122)	22.1±2.1* (136)	25.0±2.2* (154)
DNA	HP	24h	45.4±3.5 (100)	40.9±3.3* (90)	38.2±3.1* (84)	34.1±2.9* (75)	29.1±2.5* (64)
		96h	45.7±3.7 (100)	39.8±3.2* (88)	36.7±3.0* (81)	32.6±2.9* (72)	27.2±2.4* (60)
	OT	24h	53.8±4.1 (100)	46.3±3.7* (86)	43.0±3.5* (80)	38.2±3.2* (71)	33.3±3.0* (62)
		96h	52.2.0 (100)	43.8±3.7* (84)	39.7±3.3* (76)	36.0±3.1* (69)	30.3±3.0* (58)
RNA	HP	24h	35.8±3.1 (100)	31.2±3.0* (87)	29.0±2.9* (81)	25.8±2.8* (72)	21.5±2.5* (60)
		96h	34.7±3.1 (100)	29.2±2.9* (84)	25.7±2.7* (74)	22.2±2.6* (64)	18.1±2.4* (52)
	OT	24h	42.2±3.5 (100)	35.1±3.2* (84)	32.5±3.1* (77)	29.1±2.8* (69)	23.6±2.7* (56)
		96h	40.4±3.3 (100)	32.7±3.2* (81)	29.1±2.9* (72)	24.7±2.6* (61)	20.2±2.5* (50)

*, Significant ($P<0.05$), when Student's 't' test was applied between control and treated groups. Values are mean ±SE of six replicates. Values given in parenthesis are percent change with control taken as 100%.

Table 2. Total protein (TP) (μ g/mg), free amino acid (FAA) (μ g/mg) and nucleic acids (DNA & RNA) (μ g/mg) in hepatopancreas (HP) and ovotestis (OT) tissues of freshwater snail *Lymnaea acuminata* after carbaryl exposure.

	Tissue	Exposure period	Control	Carbaryl dose			
				3.0 mg/L	6.0 mg/L	9.0 mg/L	12.0 mg/L
TP	HP	24h	55.3±5.0 (100)	50.4±4.4 (91)	47.0±4.0* (85)	42.6±4.2* (77)	36.5±3.9* (66)
		96h	54.7±4.9 (100)	49.2±4.4 (90)	44.9±4.1* (82)	40.5±4.1* (74)	35.0±3.7* (64)
	OT	24h	70.6±6.5 (100)	65.7±6.2 (93)	63.6±5.8 (90)	57.2±5.6* (81)	50.9±5.7* (72)
		96h	68.5±6.1 (100)	62.3±6.0 (91)	57.5±5.9* (84)	53.4±5.4* (78)	46.6±5.0* (68)
FAA	HP	24h	17.9±2.0(100)	19.4±2.4 (108)	21.4±2.5* (119)	23.3±2.5* (130)	26.0±2.6* (145)
		96h	17.7±2.1 (100)	19.8±2.2* (112)	22.3±2.3* (126)	24.4±2.4* (138)	27.1±2.5* (153)
	OT	24h	16.4±1.7 (100)	17.8±1.8 (109)	19.8±2.1* (121)	22.1±2.2* (135)	24.0±2.3* (147)
		96h	16.3±1.6 (100)	18.4±1.8* (113)	20.8±2.4* (128)	22.8±2.4* (140)	25.9±2.5* (159)
DNA	HP	24h	45.4±3.5 (100)	39.5±3.2* (87)	36.8±3.0* (81)	31.8±2.9* (70)	26.3±2.5* (58)
		96h	45.3±3.7 (100)	38.0±3.1* (84)	34.9±3.0* (77)	28.9±2.6* (64)	23.1±2.2* (51)
	OT	24h	53.8±4.1 (100)	44.1±3.7* (82)	40.9±3.5* (76)	37.1±3.1* (69)	32.3±3.1* (60)
		96h	52.2±4.0 (100)	41.7±3.5* (80)	38.6±3.3* (74)	35.5±3.0* (68)	28.2±3.0* (54)
RNA	HP	24h	35.8±3.1 (100)	30.1±3.0* (84)	28.7±2.8* (80)	25.5±2.7* (71)	20.1±2.5* (56)
		96h	34.7±3.1 (100)	28.5±2.9* (82)	24.7±2.6* (71)	21.5±2.6* (62)	17.4±2.4* (50)
	OT	24h	42.2±3.5 (100)	33.7±3.2* (80)	31.2±3.0* (74)	28.3±2.7* (67)	22.4±2.6* (53)
		96h	40.4±3.3 (100)	31.5±3.1* (78)	28.7±2.9* (71)	23.9±2.6* (59)	19.4±2.4* (48)

*, Significant ($P<0.05$), when Student's 't' test was applied between control and treated groups. Values are mean ±SE of six replicates. Values given in parenthesis are percent change with control taken as 100%.

Table 3. Protease activity (tyrosine/mg protein/h) in hepatopancreas (HP) and ovotestis (OT) tissues of freshwater snail *Lymnaea acuminata* after dimethoate and carbaryl exposure.

Tissue	Exposure period	Control	Sub-lethal Concentration			
			3.0 mg/L	6.0 mg/L	9.0 mg/L	12.0 mg/L
				Dimethoate		
HP	24h	0.32±0.06 (100)	0.35±0.05 (109)	0.38±0.05* (118)	0.41±0.06* (129)	0.46±0.07* (145)
	96h	0.34±0.06 (100)	0.38±0.05* (112)	0.42±0.06* (124)	0.46±0.07* (136)	0.52±0.09 (152)
OT	24h	0.29±0.05 (100)	0.32±0.05* (111)	0.35±0.06* (120)	0.38±0.06* (132)	0.43±0.07* (149)
	96h	0.30±0.08 (100)	0.34±0.06* (114)	0.38±0.07* (126)	0.42±0.08* (139)	0.47±0.06* (156)
				Carbaryl		
HP	24h	0.32±0.06 (100)	0.36±0.05* (111)	0.39±0.06* (121)	0.42±0.07* (132)	0.49±0.08* (153)
	96h	0.34±0.06 (100)	0.38±0.06* (113)	0.43±0.07* (126)	0.47±0.07* (137)	0.53±0.09* (155)
OT	24h	0.29±0.05 (100)	0.33±0.04* (114)	0.36±0.05* (125)	0.39±0.06* (135)	0.44±0.07* (151)
	96h	0.30±0.08 (100)	0.35±0.05* (117)	0.38±0.06* (128)	0.43±0.07* (142)	0.48±0.08* (159)

*, Significant ($P<0.05$), when Student's 't' test was applied between control and treated groups. Values are mean ±SE of six replicates. Values given in parenthesis are percent change with control taken as 100%.

ovotestis tissue after exposure to 24h and 96h against dimethoate and carbaryl. Data also clearly shows that protease activity was increased after the exposure in hepatopancreas and ovotestis tissues. Activity of protease was maximally increased in ovotestis tissue after exposure to 24h and 96h against dimethoate and carbaryl.

Proteins are mainly involved in the architecture and physiology of the cell. During chronic periods of stress they are also a source of energy (Umminger 1977). Behavioural responses of snails exposed to sub-lethal concentration of pesticides showed that they were stressed. During stress, the snails needed more energy to detoxify the toxicants and to overcome stress. Since snails have a small amount of carbohydrates, the next alternative source of energy to meet the increased energy demand is protein. The depletion of the protein fraction in hepatopancreas and ovotestis tissues may have been due to their degradation and possible utilization for metabolic purposes. Singh et al. (1996) have also reported decline in protein constituent in different fish tissue exposed to sub-lethal concentrations of pesticides.

Increment in free amino acids level was the result of breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis (Singh et al. 1996). It also attributed to lesser use of amino acids (Seshagiri et al. 1987) and their involvement in the maintenance of an acid-base balance (Moorthy et al. 1984). Stress conditions induce elevation in the transamination pathway (Natarajan 1985). The enzyme protease functions in hydrolyzing proteins to free amino acids and small peptides. The increase in protease activity corresponds with the enhancement in the amino acid level of the tissue. Similar increase in protease activity was reported by several workers in various animals including mammals (Milliward 1970; Kabeer et al. 1984). Inhibition of RNA synthesis may also affect protein and amino acid levels. Inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. Dimethoate and carbamate appear as potential inhibitors of DNA synthesis, which might result in reduction of RNA level. Because of the electrophilic nature, these pesticides may attack many enzymes responsible for normal metabolism. Thus, it is possible that the enzyme necessary for DNA synthesis might have been inhibited by these pesticides. On compilation of the results, it appears that the damaged machinery of DNA synthesis might have affected RNA synthesis and consequently protein synthesis. So all the biochemical data indicate that dimethoate and carbaryl pesticides disturbed protein metabolism in the body tissues of the freshwater snail *Lymnaea acuminata*.

Acknowledgment. We thank the Council of Science and Technology, Uttar Pradesh for financial assessment during this work.

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