Bull. Environ. Contam. Toxicol. (1988) 40:212–218

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity of Endosulfan to Crabs: Effect on Hydromineral Balance

K. Rajeswari, V. Kalarani, D. C. Reddy, and R. Ramamurthi

Pesticide and Industrial Toxicology Centre, Department of Zoology, Sri Venkateswara University, Tirupati-517 502, India

The use of endosulfan (Thiodan (R) 6,7,8,9,10-hexachloro 1,5,5a,6, 9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3 oxide)-a broad spectrum nonsystemic insecticide-has been on the increase in India during the recent past consequent upon the ban on endrin and the decline in the use of other organochlorine pesticides due to their longer persistence and higher toxicity to mammals. Endosulfan is reported to be a fish toxicant (French et al. 1957) and its toxicity to several freshwater and marine fish has been studied (Currier 1960; Maier-Bode 1968; Herzel and Ludeman 1971; Macek et al. 1976; Reddy and Gomathy 1977; Rao and Murthy 1982; Rangaswamy 1985). Relatively little is known about the toxicity of endosulfan to many freshwater invertebrates. Though a few reports are available on the toxic effects of endosulfan on the whole animal oxygen consumption (Subhadra Devi 1985); carbohydrate metabolism (Rajendraprasad Naidu et al. 1986) and oxygen transport property of the haemocyanin (Vijayakumari et al. 1987) of the freshwater field crab, Oziotelphusa senex senex- another nontarget organism of aquatic ecosystems-the acute toxic effects of this organochlorine compound in freshwater crabs are yet to be fully elucidated. This paper presents the results of acute toxic effects of endosulfan on hydromineral balance in O. senex senex.

MATERIALS AND METHODS

Freshwater field crabs O. senex senex were collected prior to spraying operations from local paddy fields under ground (well) water irrigation to ensure that the fields from which crabs were collected were not previously contaminated. Only crabs in the weight range of 32±1 g were acclimated to laboratory conditions for about a week during which time they were fed with ad libitum minced meat. The water used in laboratory acclimation of crabs was clear, unchlorinated groundwater pumped from a deep well within the campus. The animals were not fed for 1 day before commencing the experiment to overcome differences, if any, due to differential feeding. Technical grade endosulfan (99%) dissolved in acetone was used. Sublethal (6.2 mg/L) and lethal (18.62 mg/L=96 h LC_{50}) concentrations were chosen to study acute toxic

Send reprint requests to D.C.Reddy at the above address.

effects. Uninjured, intermoult (Stage 4) crabs were separated from laboratory crab tanks and divided into three batches of six each. The first two batches of crabs were exposed for 96 h to sublethal and lethal concentrations of endosulfan respectively and the third maintained in freshwater for 96 h served as control. Acetone was added to the medium containing control animals in an amount equal to the largest aliquot (1.6 mL) of stock used in the experimental batches.

The total body weight of crabs was determined in the following manner. Crabs were taken out of the water and numbered. Then adhered water was gently wiped off with a filter paper without causing any damage. Then they were transferred to a clean, dry, preweighed corning beaker and its weight was recorded to the nearest milligram in a high precision Sartorius balance. The fraction resulting from subtraction of the weight of the beaker from the weight of the beaker plus crab gives the weight of the crab. The difference in the weight of crabs before and after exposure gives the change in the total body weight.

The total body water content (hydration level) was determined in the following manner. Crabs were taken out of the troughs and adhered water was wiped off gently with a filter paper and their weights were recorded (wet weight). Immediately they were placed in prenumbered corning beakers; allowed to dry at 100°C for 48 h and quickly transferred to a desiccator. After half hour they were taken out of the desiccator and weighed again (dry weight). The difference between the wet weight and the dry weight gives the water content. The same procedure was followed for determining the water content of the haemolymph, the claw muscle and the hepatopancreas.

The haemolymph volume was determined by the method of Lee (1961). The dye used was 0.25% congo red in 0.9% sodium chloride solution. Ten minutes after injection of 100 µL of the dye, the haemolymph was drawn and absorption of the dye in the haemolymph was measured. Haemolymph volume was calculated using the following formula

$$V = Y(dg_1/g_2)-a$$

where "g_1" and "g_2" are the amount of the dye injected and the amount of the dye in the sample respectively; "d" is the volume of the sample and "a" is the volume of saline injected with the dye.

The concentrations of sodium and potassium were estimated by flame photometric method as described by Oser (1965). A known volume of the haemolymph was deproteinized with 10% TCA, whereas known amounts of the claw muscle and the hepatopancreas were wet ashed in 3:2 (v/v) concentrated nitric acid : perchloric acid. The contents were centrifuged at 2000 g for 15 min and a clear supernatant was obtained. After making appropriate dilution, the supernatant was used for the estimation of sodium and potassium and their concentrations were calculated using calibration curves.

The concentration of chloride was estimated by the method of Robertson and Webb (1939). Tissues were deproteinized with 10% TCA and centrifuged at 2000 g for 15 min to obtain a clear supernatant. 1 mL of this supernatant was made upto 60 mL with glass distilled water. To this 5 mL of 23% phosphoric acid and 0.2 g of silver iodate were added followed by a few drops of caprylic alcohol. The contents were made up to 100 mL with glass distilled water and filtered through Whatman No.1 filter paper. To 20 mL of the clean filtrate 2 mL of saturated iodine solution were added and the liberated iodine was titrated against 0.1 N sodiumthiosulfate solution using starch as an idicator.

The significance of the data was assessed through student's "t" test (Pillai and Sinha 1968).

RESULTS AND DISCUSSION

From the results presented in Table 1, it is clear that there was a significant increase in the total body weight of crabs upon sublethal exposure. It has been reported that there was a significant drop in the major tissue organic constituents of O. senex senex exposed for 96 h to sublethal and lethal concentrations of endosulfan (Subhadra Devi 1985). This might be taken to indicate that tissue organic constituents may not contribute towards the increase in the total body weight of crabs upon sublethal exposure. Hence water, which constitutes about 80% of the cell mass, might be responsible for the increase observed in the total body weight of crabs upon sublethal exposure. Accordingly significant increases observed in hydration levels of the whole animal, the haemolymph, the claw muscle and the hepatopancreas (Table 1) support the fact that water plays a major role in increasing the total body weight of crabs upon sublethal exposure. Further there was a marked increase in haemolymph volume of crabs upon sublethal exposure, once again indicating the contributory role of water. One plausible explanation that can be put forward to explain the increase in tissue hydration levels and thereby in the total body weight was that crabs might have enhanced the rate of water uptake and water retentivity upon sublethal exposure. However, the exact reason for such an increase is yet to be clearly established. Conversely, there was a significant decrease in the total body weight, in hydration levels of the whole animal, the haemolymph, the claw muscle and the hepatopancreas and in haemolymph volume of crabs upon lethal exposure (Table 1). It has been observed that crabs upon lethal exposure showed loss of motility, inactiveness, copious mucous exudation and cessation of tactile responses, all indicating a state of pathology. As a result of this, all physiological processes including water uptake and water retentivity might have been disrupted, and this could be a plausible explanation for the loss of body weight and drop in hydration levels upon exposure to lethal concentration.

It has been stated that inorganic electrolytes like sodium and potassium are, by far, the most important substances influencing both the distribution and the retention of body water (Tyler 1975).

Table 1. Variations in the total body weight, hydration levels of the whole animal, the haemolymph, the claw muscle and the hepatopancreas, and haemolymph volume of the freshwater field crab, O. senex senex exposed for 96 h to sublethal and lethal concentrations of endosulfan. Values are mean ± SD of six individual observations. All differences are significant at 0.05 level.

Parameter	Control	Experimental	
		Sublethal	Lethal
Total body weight (g wet weight)	32.87 ± 1.53	34.27 ± 1.68	28.19 ± 1.56
% Change	-	4.26	-14.23
Whole animal hydration level (per cent water content)	86.38 ± 2.73	93.46 ± 4.82	72.63 ± 4.67
% Change	-	8.19	-15.92
Haemolymph hydration level (per cent water content)	82.58 ± 1.11	86.08 ± 1.19	70.72 ± 3.55
% Change	-	4.24	-14.11
Claw Muscle hydration level (per cent water content)	71.84 ± 1.37	74.93 ± 1.66	65.93 ± 1.22
% Change	-	4.30	- 8.23
Hepatopancreatic hydration level (Per cent water content)	76.37 ± 1.18	79.16 ± 1.12	68.46 ± 1.36
% Change	-	- 3.65	-10.36
Haemolymph volume (Volumes per cent)	42.69 ± 1.73	46.78 ± 2.24	30.77 ± 2.56
% Change	-	9.58	-27.92

The concentrations of these osmotically effective electrolytes would be expected to vary when there is a change in body water content. As such the results presented in Table 2 clearly show, there was a marked decrease in the concentrations of sodium, potassium and chloride in the haemolymph, the claw muscle and the hepatopancreas of crabs upon exposure to both sublethal and lethal concentrations of endosulfan (Table 2), indicating osmotic imbalance since maintenance of osmoregulation involves these inorganic osmoeffectors. Observations of changes in the total body weight and tissue hydration levels are of value in differentiating decrease in sodium and potassium due to dilution resulting in weight gain from those in which true depletion of sodium and potassium has occurred resulting in weight loss due to dehydration.

Table 2. Variations in the concentrations of sodium, potassium and chloride in the haemolymph (HL), the claw muscle (CLM) and the hepatopancreas (HP) of the freshwater crab, O. senex senex exposed for 96 h to sublethal and lethal concentrations of endosulfan. Values are mean ± SD of six individual observations. All differences are significant at 0.05 level.

Parameter	Control	Experimental	
		Sublethal	Lethal
HL Sodium (m mol/L)	352.45 ± 26.83	326.57 ± 22.83	305.46±27.4
% Change	-	- 7.34	-13.33
CLM Sodium (m mol/g wet weight)	45.63 ± 1.67	42.87 ± 1.06	40.61±1.33
% Change	-	- 6.05	-11.0
HP Sodium (m mol/g wet weight)	33.76 ± 1.12	31.72 ± 1.45	28.44±1.08
% Change	-	- 6.04	-15.75
HL Potassium (m mol/L)	10.28 ± 0.94	9.40 ± 0.713	8.64±0.034
% Change	-	- 8.56	-15.95
CLM Potassium (m mol/g wet weight)	84.77 ± 2.53	79.76 ± 3.15	76.53±3.32
% Change	-	- 5.91	- 9.72
HP Potassium (m mol/g wet weight)	73.88 ± 1.48	70.87 ± 1.82	66.74±1.52
% Change	-	- 4.07	- 9.66
HL Chloride (m mol/L)	204.91 ±17.89	183.99 ±16.33	177.45±18.55
% Change	-	- 10.21	-16.33
CLM Chloride (m mol/g wet weight)	112.44 ± 4.75	102.55 ± 4.97	95.35± 4.26
% Change	-	- 8.80	-15.20
HP Chloride (m mol/g wet weight)	85.43 ± 3.13	81.66 ± 3.83	74.56± 3.52
% Change	-	- 4.41	-12.72

Accordingly, the decrease noticed in sodium, potassium and chloride (both the intake and output of chloride are, in fact, inseparable from those of sodium) in the haemolymph, the claw muscle and the hepatopancreas of crabs exposed to sublethal concentration of endosulfan need not necessarily indicate true loss of these

ions since there was a significant increase in the total body weight, haemolymph volume and hydration levels of the whole animal, the haemolymph, the claw muscle and the hepatopancreas of crabs upon sublethal exposure. As such, the decrease in these inorganic cations upon sublethal exposure could be attributed more to dilution rather than to their true loss from the body. On the other hand, the decrease in these inorganic cations upon lethal exposure could be the result of a true loss since lethal exposure is manifested by rapid weight loss and a significant decrease in hydration levels of the whole animal, the haemolymph, the claw muscle and the hepatopancreas (Table 1). Based on the above observations, it is likely that in crabs exposed to sublethal concentration of endosulfan the decrease in tissue sodium, potassium and chloride could be an effect of overhydration and that the decrease upon lethal exposure could be an effect of loss of body water representing a true loss.

REFERENCES

Currier JP (1960) Preliminary report on experimental reclamation with Thiodan. Proc Mid-West Fish Wildl Conf, Toronto, 5-7 December 1960, 5pp

French H, Goebell H, Standerman W, Finkenbrink W (1957) Unsaturated polycyclic sulfites and a process for their manufacture. US Patent office, Patent No 2. 799, 685

Herzel F, Ludemann D (1971) Behaviour and toxicity of endosulfan (Thiodan) in water under different experimental conditions. Z angew Zool 58:57-61

Lee HM (1961) The variation of blood volume with age in the desert locust, Schistocerca gregaria (Forsle). J Insect Physiol 6 (1):36-51

Macek KJ, Lindberg MA, Scott S, Bauston KS, Costa PA (1976) Toxicity of four pesticides to water fleas and fathead minnows. Acute and chronic toxictiy of acrolein, heptachlor, endosulfan and trifluralin to the water flea (Daphnia magna) and fathead minnow (Pimephales promeles) US Environmental protection Agency (EPA-600/3-76-099)

Maier-Bode H (1968) Properties, effect, residues and analytics of the insecticide endosulfan. Residue Rev 22:1-44

Oser BL(1965) Hawk's physiological chemistry, 14th ed.McGraw Hill Book Co., New York

Pillai SK, Sinha HC (1968) Statistical methods for biological workers. Ramprasad and Sons, Agra, India

Rajendra Prasad Naidu K, Reddy DC, Naidu BP (1986) Changes in certain aspects of carbohydrate metabolism in tissues of the fresh water field crab, Oziotelphusa senex senex (Fabricius) during endosulfan stress. Indian J Exp Biol 24:797-798

Rangaswamy CP (1985) Impact of endosulfan toxicity on some physiological properties of the blood and aspects of energy metabolism of a fresh water fish, Tilapia mossambica (Peters) PhD Thesis S.V. University, Tirupati, India

Rao DMR, Devi AP, Murthy AS(1982) Toxicity and metabolism of endosulfan in three freshwater cat fishes. Environ Pollut (Ser A) 27:223-231

Reddy TGK, Gomathy S (1977) Toxicity and respiratory effects of pesticide Thiodan on catfish, Mystus vittatus. J Environ Hlth 19:361-3

Robertson JD, Webb DA (1939) The microestimation of sodium, potassium, calcium, magnesium, chloride and sulphate in seawater and body fluids of marine animals. J Exp Biol 16:155-177

Subhadra Devi G (1985) Impact of endosulfan toxicity on whole animal oxygen consumption and biochemical composition of the haemolymph of the freshwater field crab Oziotelphusa senex senex (Fabricius).M Phil dissertation S.V. University, Tirupati, India

Tyler DD (1977) Water and mineral metabolism. In Harper HA, Rodwell VW, Mayer PA (eds) Review of Physiological Chemistry. Lange Medical Publications, Maruzen Co., California. p 516

Vijayakumari P, Reddy DC, Ramamurthy R (1987) Acute toxicity of endosulfan to crab: Effect on transport property of haemocyanin. Bull Environ Contam Toxicol 38:742-747

Received April 27, 1987; accepted October 31, 1987