Bull. Environ. Contam. Toxicol. (2001) 66:269–276
© 2001 Springer-Verlag New York Inc.
DOI: 10.1007/s001280000234

Bulletin of Environmental Contamination and Toxicology

Deltamethrin, Effects on Volume Control, and Water Balance in *Eisenia fetida* Sav. (Annelida, Lumbricidae)

E. Fischer,[1] A. Tóth,[1] S. Farkas[2]

[1] Hungarian Academy of Sciences, University of Pécs, Adaptation Biology Research Group, and Department of General Zoology and Neurobiology, University of Pécs, H-7624 Pécs, Ifjúság u. 6, Hungary
[2] Department of Zootaxonomy and Synzoology, University of Pécs, H-7624 Pécs, Ifjúság u. 6, Hungary

Received: 31 May 2000/Accepted: 11 October 2000

Deltamethrin belongs to the α-cyano group possessing type II pyrethroid insecticides. Pyrethroid insecticides may accumulate by 8- to 30-fold bioconcentration factors in earthworms (Curl et al 1987). Because of the high accumulation rate and known neurotoxic activity (Narashi 1994) Kiss and Tóth (1999) examined in our laboratory the long-term effect of deltamethrin on the moving and feeding activity of juveniles of *E. fetida*. They found that 25 mg/kg deltamethrin content in the culture media prevents the growth of the juveniles; moreover, greater concentrations may induce a marked weight loss in the first week of treatment. The loss of weight as an effect of pyrethroid insecticides is consistent with the findings of Cigli and Jepson (1995) and Tan (1981). They concluded that weight loss in butterfly larvae is a consequence of feeding inhibition. The main effect of 50 mg/kg deltamethrin found in our preliminary experiment was a more than 40 % weight loss within one week of treatment. However, not even starved earthworms suffer such a rapid and high weight loss in our experiments, therefore anorexia may be excluded as a reason for the weight loss.

In order to examine the reason for the deltamethrin-induced short-term weight loss in earthworms we used a modified contact filter-paper test method (OECD 1984) in this work. We postulated that the altered salt and water balance may the true reason for the deltamethrin induced rapid weight loss. Therefore, the proportion of the dry mass/water content of control and of deltamethrin-treated earthworms was also examined. The relationship between osmoregulation (electrolyte and water balance) and volume control is evident (Boroffka 1968, Stephenson 1945). Transfer of earthworms to high salinity resulted in an initial weight loss due to osmotic water efflux, which is not usually regained even after long periods of time in the higher salinity (Oglesby 1978). In this work the effect of hyperosmotic NaCl on the body weight of earthworms was also established and compared with those of deltamethrin.

Type II pyrethroids greatly prolong the open time of neuronal voltage-dependent sodium channels (Narashi 1994, Vijverberg and van den Brecken 1990) resulting in CS (choreoanthetosis, salivation) syndrome in mammals.

Correspondence to: E. Fischer

Neurohormones of the earthworm brain influence the water and ion balance (Carley 1978a, 1978b, Kamemoto 1964, Zimmermann 1973). It may therefore be supposed that a neuronal or neurohormonal mechanism may mediate the effect of deltamethrin. Comparison of the reactions of intact and decapitated earthworms served in this work for proving or disproving this supposition.

The earthworm's body wall, gut and nephridia participate in the maintenance of hyperionic steady-state and osmotic equilibrium of body fluids (Oglesby 1978). Active transepithelial Na^+ and Cl^- transport through the body wall epidermis has been proved, but the mechanisms are independent of each other (Dietz and Alvarado 1970, Dietz 1974). Sodium absorption of the epidermis may be inhibited by the diuretic drug amiloride (Kischner 1979, Prush and Otter 1977). Amiloride-sensitive Na^+ transport is well known in the Na^+-reabsorbing epithelia of both vertebrates and invertebrates (Gerencser et al. 1999, Horisberger 1998, Onken 1999), as well as in the body wall epithelium (Prush and Otter 1977) and in the gut epithelium (Cornell 1984) of earthworms. In this work the effect of amiloride on the body weight of earthworms was also examined.

Short-term filter paper trials were projected for study of the kinetics and mechanism of deltamethrin-induced weight alterations of *E. fetida*. We studied the several-hour and one-day effect of deltamethrin on the body weight of earthworms and on the water content of their body. Decapitated earthworms served for examination of the supposed mediating role of cerebral neurosecretions in the desiccation process. For comparison, we studied the effect of hyperosmotic NaCl and of amiloride on the weight alterations. Results prove that deltamethrin causes a marked and non neurohormon-mediated water loss in *E. fetida*.

MATERIALS AND METHODS

Specimens of *Eisenia fetida* (Savigny) were obtained from the stock culture in our laboratory. Juveniles (without clitellum) were placed into plastic bags with moist filter paper on the bottom for 36-48 hours. In this way, the gut content was avoided and the weight of the worms could be stabilized for several days. The temperature of the laboratory was 23°C. At the start of the experiments, the worms were placed between two filter papers which rapidly absorbed the water, and weighed with an Ohaus-type analytical balance.

The experiments were carried out in 200 cm^3 plastic bags. The bottoms and sides of the bags were lined with filter paper moistened with a solution containing the desired test substance (OECD 1984). Worms were weighed, and 10 specimens were introduced to each bag. They were weighed again at 3, 24, and 72 hours after the start of experimental treatment. Untreated controls kept on filter paper moistened with tap-water were used for each series.

Deltamethrin stock solution was made with distilled water from the insecticide DECIS 2,5 EC (Hoechst Schering AgrEvo). In the first series of the experiments stock solution was diluted before the treatment with tap-water for suspensions containing 100, 50, 25, and 12.5 mg/L active ingredient. Three repeats were made in each concentration. Dry masses of treated worms and controls were determined. After the treatment, worms were dried at 105°C until reaching constant weight.

In the second series of experiments decapitated animals were used. The cerebral and hypopharyngeal ganglia were removed by decapitation. The worms were then kept for 10 days in wet chambers for wound healing before the experimental treatment with deltamethrin.

For comparison of the effects of deltamethrin with substances known for their effects on the salt and water balance of earthworms, NaCl and amiloride were used in the third series of experiments. Filter paper moistened with 1 % NaCl was used for hyperosmotic treatment. Amiloride HCl (SIGMA) was similarly applied, but in 1000, 500, 250, and 100 mg/L final concentrations.

For statistical evaluation the ANOVA-Tukeys-test of the Toxstat 3.3 program (Gulley et al. 1988) was used. Significance was determined at $p < 0.05$, and was evaluated and marked in the tables in each experimental series and in each treatment time only in comparison to the own original weight of the experimental animals.

RESULTS AND DISCUSSION

The weight of the control worms did not change significantly during the experiments. However, exposure to deltamethrin produced a very rapid and marked decrease in the body weight (Table 1).

Table 1. Effect of deltamethrin on the body weight (mg) of *E. fetida*.

Treatment	**Start**		**3 hr**			**24 hr**		
	mean	SD	mean	SD	$p<0.05$	mean	SD	$p<0.05$
untreated	165	40.4	157	40.8	-	158	38.6	-
deltamethrin 100 mg/L	173	25.1	142	23.3	*	141	29.3	*
deltamethrin 50 mg/L	179	35.8	119	19.5	*	120	26.3	*
deltamethrin 25 mg/L	169	38.5	109	21.5	*	104	33.1	*
deltamethrin 12.5 mg/L	164	36.1	130	34.9	-	127	35.9	-

(n = 10; * = significant difference compared with the original weight of the animals in each concentration and treatment time)

Maximum weight loss occurred after 3 hours exposure to 50 and 25 mg/L deltamethrin. In comparison with the controls, more than 30% weight loss was evoked by the deltamethrin treatment. The lower weight-decreasing effect of 100 mg/L deltametrin may be the result of the escape of worms from contact with deltamethrin by crawling to the tops of the bags. The weight loss from 12.5 mg/L deltamethrin was not significant.

The percentage dry mass of control worms was 15.6 %. After the deltamethrin-induced 19.8 % body weight loss, dry mass increased to 18.3 % (Table 2). The results prove that the rapid decrease in the body volume on the effect of deltamethrin is a consequence of the decreased water content of the body, i.e. desiccation.

Table 2. Effect of deltamethrin on the percentage dry mass of *E. fetida*

Treatment	**Dry mass %**	**SD**	**$p < 0.05$**
untreated	15.6	0.2	
deltamethrin 25 mg/L	18.3	0.7	*

(n = 3; * = significant difference compared with the control)

E. fetida respond to irritating stimuli with violent crawling, escape and coelomic fluid expression through the dorsal pores. Expressed coelomic fluid leaves yellow-brown coloration on the filter paper. On the effect of crystalline NaCl the coelomic fluid expression resulted in a 34.4 % weight loss, but after the worms were washed with tap-water and kept in a wet-box for 3 hours, the weight loss dropped by rehydration to 17.7 %. Neither deltamethrin in lower than 100 mg/l concentrations nor 1% NaCl have an irritating effect, and both resulted in a significant effect on body weight (Table 3). The effect of deltamethrin on the body weight was not reduced markedly during one week preincubation in wet filter paper at 23°C (Table 3).

Earthworms maintain an ionic steady state for a long time when living in fresh water (Dietz and Alvarado 1970). Maintained in fresh-water or in low salinity medium they are strongly hyperosmotic. As salinity increases, body fluids remain hyperosmotic to the medium, though there is hyperosmotic conformity (Oglesby 1978). However, with regard to sodium concentration, earthworms show hyper-regulation in low salinity and hypo-conformity in high salinity medium (Dietz and Alvarado 1970, Oglesby 1978). Sodium concentration in the celomic fluid of *E. fetida* living under natural conditions (in wet soil) is 79.7 mM/l, when introduced to a 100 mM/kg medium the concentration increases to 116.5 mM/l (Kamemoto et al. 1962), but over a certain limit it can not increase more. Stephenson (1945) established maximal body volume of *Lumbricus terrestris* in 0.35 % NaCl solution by short-term immersion experiments, at both lower and higher salinity the volume decreased. As a rapid response to hyperosmotic stress the body volume of fresh-water leeches shows 30-35 % decrease (Boroffka 1969, Reynoldson and Davies 1980).

Table 3. Effect of deltamethrin, preincubated deltamethrin, NaCl and amiloride on the body weight (mg) of intact specimens, and the effect of deltamethrin on decapitated specimens of *E. fetida*

Treatment	**Start**		**3 hr**			**24 hr**		
	mean	SD	mean	SD	$p<0.05$	mean	SD	$p<0.05$
deltamethrin 25 mg/L	169	38.5	109	21.5	*	104	33.1	*
NaCl 1 %	152	22.9	119	18.3	*	101	16.6	*
D-25 preincubated	181	17.8	126	17.6	*	130	14.9	*
D-25 decapitated	133	21.0	103	21.2	*	96	17.8	*
amiloride 1000 mg/L	177	42.6	159	38.2	-	146	36.5	-

(D-25= deltamethrin 25 mg/L; n = 10; * = significant difference compared with the original weight in each experiment)

Decapitation did not prevent the weight decreasing effect of deltamethrin (Table 3). The water balance and volume control mechanisms of earthworms depend on the brain neurohormones (Carley 1978a, Kamemoto 1964, Zimmermann 1973). Decapitated earthworms showed significant weight loss on the effect of deltamethrin treatment, similarly to the intact ones. Therefore, the importance of neurohormones in the deltamethrin-induced dehydration may be excluded. However, ionic balance and volume control during rehydration of desiccated earthworms may be mediated by neuro-endocrine mechanisms.

Type II pyrethroids are neurotoxic agents, modifying the sodium channel gating kinetics. The changes in sodium channel gating cause a marked membrane depolarization (Forshaw et al. 1993, Narashi 1994, Vijverberg and van den Brecken 1990). It is known that beside the nephridia (Boroffka 1968), the body wall (Dietz 1974, Dietz and Alvarado 1970, Prush and Otter 1977) and intestinal epithelia (Cornell 1984) of annelids are involved in Na^+ and Cl^- transport, and that the Na^+ and Cl^- transport mechanisms are independent of each other. We supposed that earthworm possess deltamethrin-sensitive epithelial sodium transport mechanisms. Thos, the deltamethrin-induced Na^+ (and probably also equivalent anion) loss may result in the dehydration, as an osmoregulative compensatory response to the electrolyte deficit.

To verify of this hypothesis, we used amiloride-treated earthworms (Table 4). Amiloride, a diuretic drug, is a potent inhibitor of Na^+ transport in a wide variety of systems (Kirschner 1979). Amiloride in a 1000 mg/L concentration evoked a low irritation of the worms in the first hour of treatment, but their decreasing effect on the body weight was small during the 24-hour treatment (Table 3). However, on the effect of 72-hour treatment 1000 mg/L amiloride resulted in a more than 30 % weight loss. Amiloride in 500 mg/L and 250 mg/L concentrations after 72-hour treatment also resulted in significant weight loss without irritating effect (Table 4).

Table 4. Effect of amiloride treatment on the body weight (mg) of *E. fetida*

Treatment	**Start**		**24 hr**			**72 hr**		
	mean	SD	mean	SD	$p<0.05$	mean	SD	$p<0.05$
untreated	150	40.5	151	44.0	-	146	42.3	-
amiloride 1000 mg/L	185	38.9	149	33.8	-	123	29.8	*
amiloride 500 mg/L	141	12.6	131	10.8	-	115	11.7	*
amiloride 250 mg/L	128	11.3	117	13.5	-	95	6.1	*
amiloride 100 mg/L	166	51.0	152	48.9	-	132	45.1	-

(n = 10; * = significant difference compared with the original weight of the animals in each concentration and treatment time)

Amiloride-sensitive Na^+-channel is a key element of the electrogenic sodium transport by kidney, colon and gland duct epithelia (Horisberger 1998); its role in the regulation of extracellular fluid volume has been highlighted. Amiloride-sensitive sodium transport in the body wall epithelium (Prush and Otter 1977), in the gut epithelium (Cornell 1984) of earthworms, and in epithelia of other invertebrates (Gerencser et al. 1999, Kirschner 1979, Onken 1999) has been proved. Amiloride resulted in a significant weight loss in our experiments. This supports our hypothesis that decrease of the sodium content of body fluids may trigger hyperosmotic regulation, maintained by proportional anion and water loss.

The main difference found between the effect of deltamethrin and amiloride on body weight was the rapid effect of deltamethrin and the slow effect of amiloride. Deltamethrin probably delays the closing of the epithelial Na^+ channels, therefore a rapid sodium flow may be directed toward the hypoosmic medium. Moreover, Forschaw et al (1993) concluded that chloride channels may also be inhibited by deltamethrin. In contrast, the active sodium input may be inhibited by amiloride. Consequently the slow sodium loss of the earthworms occurring in the hypoosmotic medium may not be compensated by sodium reabsorption from the surrounding of amiloride-treated earthworms.

REFERENCES

Boroffka I (1968) Osmo-und Volumen regulation bei *Hirudo medicinalis*. Z Vergl Physiol 57:348-375.

Carley W W (1978 a) Neurosecretory control of integumental water exchange in the earthworm *Lumbricus terrestris* L. Gen Comp Endocrinol 35:46-51.

Carley W W (1978 b) Water economy of the earthworm *Lumbricus terrestris* L.: Coping with the terrestrial environment. J Exp Zool 205:71-78.

Cigli T, Jepson P C (1995) The risk posed by deltamethrin drift to hedgerow butterflies. Environ Pollut 87:1-9.

Cornell J C (1984) Seasonal changes in sodium transport and amiloride sensitivity in the isolated intestine of the earthworm, *Lumbricus terrestris* (L.). Comp Biochem Physiol 78A:463-468.

Curl E A, Edwards P J, Elliott C, Leahey J P (1987) The conjugation and accumulation of metabolites of cypermethrin by earthworms. Pestic Sci 20:207-222.

Dietz T H (1974) Active chloride transport across the skin of the earthworm, *Lumbricus terrestris* L. Comp Biochem Physiol 49A:251-258.

Dietz T H, Alvarado R H (1970) Osmotic and ionic regulation in *Lumbricus terrestris* L. Biol Bull 138:247-261.

Forshaw P J, Lister T, Ray D E (1993) Inhibition of a neuronal voltage dependent chloride channel by the type II pyrethroid, deltamethrin. Neuropharmacology 32:105-111.

Gerencser G A, Kornette K M, Loo S Y (1999) Intracellular Na^+ activities in Aplysia gut: Effescts of transport inhibitors on the Na+ pump. Comp Biochem Physiol 122A:221-226.

Gulley D D, Boelter A M, Bergman H L (1988) Toxstat version 3.3. ANOVA-Tukeys Test. University of Wyoming, Laramie, WY.

Horisberger J-D (1998) Amiloride-sensitive Na channels. Current Opinion in Cell Biology 10:443-449.

Kamemoto F I (1964) The influence of the brain on osmotic and ionic regulation in earthworms. Gen Comp Endocrinol 4:420-426.

Kamemoto F I, Spalding A E, Keister S M (1962) Ionic balance in blood and coelomic fluid of earthworms. Biol Bull 122:228-231.

Kirschner L B (1979) Extrarenal action of amiloride in aquatic animals. In: Cuthbert A W, Fanelli G M Jr, Scriabine A (eds) Amiloride and Epithelial Sodium Transport. Urban and Schwarzenberg, Baltimore. pp. 41-49.

Kiss P, Tóth A (2000) Sublethal effects of deltamethrin in *Eisenia fetida* (Annelida, Lumbricidae) using laboratory breeding experiments (in Hungarian) PTE TTK, Pécs, Degree dissertation.

Narashi T (1994) Role of ion channels in neurotoxicity. In: Chang L W (ed) Principles of Neurotoxicology, Marcel Dekker Inc. New York. pp.609-655.

OECD (1984) Guideline for Testing Chemicals no 207 Earthworm, acute toxicity tests, pp 1-9. Adopted 4 April 1984, Paris.

Oglesby L C (1978) Salt and water balance. In: Mill P J (ed) Physiology of Annelids. Academic Press, London. pp. 555-657.

Onken H (1999) Active NaCl absorption across split lamellae of posterior gills of Chinese crabs (*Eriocheir sinensis*) adapted to different salinities. Comp Biochem Physiol 123A:377-384.

Prusch R D, Otter T (1977) Annelid transepithelial ion transport. Comp Biochem Physiol 57A:87-92.

Reynoldson T B, Davies R W (1980) A comparative study of weight regulation in *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea). Comp Biochem Physiol 66A:711-714.

Stephenson W (1945) Concentration regulation and volume control in *Lumbricus terrestris* L. Nature 155:635.

Tan K (1981) Antifeeding effect of cypermethrin and permethrin at sub-lethat against *Pieris brassicae* larvae. Pestic Sci 12:619-626.

Vijverberg H P M, van den Brecken J (1990) Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit Rev Toxicol 21:105-126.

Zimmermann P (1973) Zur neuronalen Regulation des Wasserhaushalts bei *Lumbricus terrestris* L. Z Zellforsch 145:103-118.