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Short Communication

Importance of Dose Metrics for Understanding Sublethal Pentachlorophenol Toxicity in Aquatic Systems

Olli Pekka Penttinen[1]* and Jussi VK Kukkonen[2]

[1]*University lecturer, Ecosystems and Environment Research Programme, University of Helsinki, Finland*

[2]*Professor, Department of Environmental and Biological Sciences, University of Eastern Finland, Finland*

Corresponding author: Olli-Pekka Penttinen, Ecosystems and Environment Research Programme, University of Helsinki, Finland.

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Abstract

Whole animal metabolic rate as mechanism-specific response metrics related to respiratory uncoupling represents sensitive body residue approach that bridges ecotoxicology with ecophysiology. In this context more information is required for understanding the coupling of metabolic rate affecting factors, their directions and magnitude as a functional component of the organism's metabolic machinery. Here environmental temperature was relevant source of variability to be explored and form the focus of this study. The direct calorimetry was used to measure metabolic rate of oligochaete worm *Lumbriculus variegates*, and effect of a known uncoupling agent pentachlorophenol (PCP) in acute chemical exposures at low or low and high temperatures (2 and 20 ºC for *L. variegatus*) was monitored.

The data showed many important accumulation patterns of PCP for aquatic worm. As a result, the highest BCF value of ca. 1300 was found in the worms exposed to PCP concentration at 20ºC. The test organisms responded to treatments of PCP with a significant dose dependent increase in heat output by a maximum factor of three. We conclude that the body residue approach makes a Transition from Toxicology to Ecotoxicology by forming a linkage to abiotic and biotic factors that modify bioavailability, bioaccumulation, and toxic response.

Keywords: Body residues; Direct calorimetry; Ecotoxicology; *Lumbriculus variegatus*; Metabolic rate; Pentachlorophenol; Physiological trait; Pentachlorophenol

Introduction

Body residues with molar units have high toxicological relevance and the relationship between body residues and biological effects, including both lethal or sublethal effects, is of ecotoxicological importance especially helping to overcome difficulties related to bioavailability in different environmental compartments [1-3] by reducing variability in toxicity data [4].

In our previous studies we have used metabolic rate of aquatic organisms as a response metrics with the aim of determining body residues for chemicals acting as uncouplers of oxidative phosphorylation.

The results indicate that an acute exposure to uncoupling agents leads to dose-depending metabolic rate-enhancing effect at the whole organism level in different aquatic species under variable environmental conditions, if a critical internal threshold concentration for response development is achieved [5,6].

In this context more information is required for understanding the coupling of metabolic rate affecting factors, their directions and magnitude as a functional component of the organism's metabolic machinery. From our perspective, environmental temperature is important since the ambient environmental temperature represents the most important external factor affecting metabolic rate. As a result, the initial change of metabolic rate in poikilotherms increases or decreases by a factor 2-3 per 10°C change within the thermal range they are able to get adapted [4]. At the same, 1.5 to 3-fold increase in overall metabolic rate is reported in aquatic organisms exposed to uncoupling agents [6,7].

Here environmental temperature was a relevant source of variability to be explored in the context given above and form the main focus of this study. Thus, metabolic rate of oligochaete worm Lumbriculus variegatus was measured, and effect of a known uncoupling agent pentachlorophenol (PCP) in 24h chemical exposures at two temperatures (2 and 20ºC) on metabolism was analyzed.

Material and Methods

The oligochaete worm L. variegatus was reared in the Aquatic Toxicology Laboratory at the University of Eastern Finland under conditions previously described [6]. The cold-acclimated culture (3±1°C) was formed three years before use in the experiments. The unlabeled PCP; originally 98% pure was obtained from Fluka (Buchs, Switzerland) and [14C]-labeled pentachlorophenol (PCP); specific activity 6.5 µCi/mmol from Sigma Chemical (St. Louis, MO, USA).

Two sets of experiments were run with L. variegatus; one set at 2°C, and another one at 20°C. At both temperatures, animals were exposed to a mixture of radiolabeled and nonlabelled PCP for 24 h in the beakers including controls and seven test concentration that ranged from 0.08 to 1.4 µM, and from 0.08 to 0.8 µM, for 2 and 20°C, respectively. During the last two exposure hours, animals were incubated in the closed microcalorimetric ampoule while their heat dissipation was continuously monitored.

The metabolic rate of individual test organisms was monitored directly as the rate of heat dissipation (=heat output) by using an isothermal heat conduction type microcalorimeter TAM 2277 (Thermal Activity Monitor, Thermometric, Järfälla, Sweden). The post-measurement treatments of samples, radioactivity measurement, heat output data acquisition and statistical analysis were done as described previously [5,6].

A bioconcentration factor (BCF) on a wet mass basis was calculated as the ratio of concentration in organisms after 24h (L. variegatus and the corresponding chemical concentration in the surrounding water; BCF= CB/CW (µmol/kg / µmol/ L).

Results and Discussion

After a 24-h exposure, the worms accumulated PCP body residues in the range of 0.045 to 0.66 (*L. variegatus* at 2°C) and 0.11to 0.64 (L. variegatus at 20°C) µmol/g wet weight (Table 1). This accounts for 1.7 to 6.3% (mean ± standard deviation [SD] 4.1± 1.1), 2.6 to 10.4 % (5.6± 1.6), of initially added PCP for L. variegatus at 2 and 20°C.

Table 1: Treatment-specific information of body size (wet mass), metabolic rate (µW/g), pentachlorophenol body residues (µmol/g), and bioconcentration factors for *Lumbriculus variegatus* (Lv) at two temperatures.

Lv 2ºC	Body mass		Heat output			% of	Body residues				
Concn µM/L	mg	±SD	µW/g	±SD	Sg.	control	µmol/g	±SD	BCF	±SD	n
Control	8.08	± 3.02	0.163	±0.014	A	-	-	-	-	-	21
0.08	8.55	± 1.52	0.168	±0.030	AB	104	0.046	± 0.008	582	±113	8
0.16	7.04	± 0.99	0.166	±0.031	AB	102	0.087	±0.016	533	±98	8
0.3	6.67	± 0.65	0.219	±0.036	B	135	0.19	±0.015	658	±52	7
0.42	8.54	± 1.30	0.269	±0.040	C	166	0.253	±0.022	591	±55	8
0.75	7.4	± 1.62	0.314	±0.032	D	194	0.389	±0.032	532	±57	8
1.15	7.19	± 1.52	0.351	±0.048	D	217	0.521	±0.027	470	±64	5
1.5	6.1	± 1.67	0.34	±0.061	D	210	0.658	±0.028	465	±23	5
Lv 20°C											
Control	6.83	± 2.86	0.96	±0.207	A	-	-	-	-	-	24
0.08	5.27	± 0.48	1.147	±0.207	AB	119	0.106	±0.017	1273	±23	8
0.15	7.93	± 1.19	1.309	±1.187	C	136	0.156	±0.011	1025	±23	8
0.33	4.6	± 0.47	1.683	±0.318	D	175	0.341	±0.026	1000	±33	7
0.38	5.36.	± 1.38	2.06	±0.327	E	215	0.366	±0.046	971	±46	8
0.54	5.98	± 1.89	2.414	0.345	F	251	0.577	±0.038	806	±19	8
0.78	4.66	± 1.35	2.248	0.328	F	234	0.641	±0.085	821	±22	6

[ab] Significance; treatments with the same uppercase letter are not significantly different from one another (ANOVA , α=0.05), cHeat output value as percentage of the relevant control

The observed body residues were significantly different among temperature ($F_{1,108}$= 9.46, p= 0.003). As a result, BCFs (Table 1) varied a hundredfold between the treatments, and the highest BCF value of ca. 1300 was found in the worms exposed to PCP concentration of less than 0.4 µM at 20ºC. Above internal PCP threshold concentration of ca. 400 µmol/g gradual decrease of the BCF in the worms was observed.

At 2°C, heat output of unexposed worms varied from 0.91 to 2.1 µW/ individual (mean ±SD 1.26 ± 0.35, n=21) or 90.8 -196.6 µW/g (162.6 ± 14.3). The heat output showed an increased rate at a higher exposure temperature with the temperature coefficient Q10 of 3.1 (L. variegatus; control rate). At 20ºC, the highest individual level variation among treatments was recorded (2.8 to 16.8 µW/ individual (mean ±SD; 7.3± 3.8, n= 24) but it was reduced by using mass-scaled rate that has a range from 627.0 to 1355.0 µW/g (959.7 ± 207.0). At both temperatures after 24h PCP exposure, the test organisms responded to treatments of pentachlorophenol with a significant dose dependent increase in heat output by a maximum factor three for *L. variegatus*.

That physical, chemical, and biological factors affect metabolic rates has been known for a long time, but information about toxicant-induced variation in metabolism is limited although it is expected that within aquatic systems and organisms there will be variability between pollutant-exposed individuals in terms of physiological performance. That acute exposure to pentachlorophenol leads to a heat output-enhancing effect has also been known for a long time and therefore PCP is an appropriate model compound both in terms of physiological response [5-7] and the body residue-based approach [5-6]. Weak acid respiratory uncouplers, such as pentachlorophenol, exert their toxicity by disrupting the electrochemical proton gradient built up across the inner mitochondrial membrane. This leads to increased oxygen consumption and metabolic heat output. The current study observed that exposure to PCP significantly increased the heat output of exposed organisms. An attempt to form causal heat output-mediated link between tissue residues and PCP toxicity was also successful. Consequently, a direct link to the body residue concept i.e., link between bioaccumulation and ecotoxicity, was established.

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Conflict of Interest

None.

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