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Phenol Accumulation in the Larvae of the Species *Agrion puella* L. (Insecta, Odonata) under Laboratory Conditions

Summary: In an aerated batch experiment for one to six days the phenol accumulation is investigated in the surviving and killed larvae of *Agrion puella* at phenol concentrations of 50 to 200 mg/l in the medium. The rate of mortality increases with the initial concentration of phenol, although about 40 % phenol is microbially digested during the test. The phenol accumulation in $\mu\text{g/g}$ wet substance is always higher in the surviving individuals than in the killed ones, but it shows an identical time trend for both. On the first day, accumulation is high, and till the third day it decreases. On the fourth day, it reaches a maximum, and then it drops sharply to its minimum on the sixth day. The measured actual accumulation is the result of changing intake and elimination rates, especially the elimination rates decreasing with increasing duration of tests and test concentration due to metabolic disturbances. The absolute minimum on the sixth day is determined also by the reduced phenol concentration. The limit of accumulation for a chronic poisoning can be estimated at 70 $\mu\text{g/g}$ phenol.

Introduction

The researches on the phenol accumulation in the larvae of the *Agrion puella* L. are an extension of the former researches connected with this problem (ERBEN et al.). By taking this species we tried to extend such information to a great part of different groups of invertebrates. We hoped thus to get some picture of the manner of the function of this poison after its accumulation inside the organism.

The data available in connection with the effect of phenol (BACH; LIEBMANN; ALEKSEEV and USPENSKAJA; and many others) have been applied to this species too, which, for the greater part, showed a specific behaviour. The recent experience enabled our better work and an easier interpretation of results with *A. puella*.

Material and Methods

The animals were gathered in the Peščenka brook, a tributary stream of the Odra river. After bringing them into the laboratory, we put them in glass tubes, 10 individuals each, filled with tap-water. The animals were left for 24 h to adapt to the laboratory conditions. After that we started adding phenol concentrations of 50 to 200 mg/l in portions of 25 mg/l. One glass tube was left as a control set. The animals were not fed. All tubes were aerated. The experiments lasted for 6 d and were repeated three times.

Besides the quantity of the accumulated phenol the following parameters were measured: water temperature, dissolved oxygen, total hardness, the titer of the saprophytic bacteria and the phenol quantity in the experimental sets.

The quantity of the accumulated phenol was measured in the alive as well as in the dead individuals on each day of the experiment and was expressed in microgrammes per gramme of bodyweight. The dead and the alive individuals were dried with filter-paper, weighed and then put for cooking in 100 ml of aqua dest., where — with the aid of phosphoric acid —, pH had been set at 4, along with the indicator methyl-orange.

All parameters were measured by the APHA methods.

Results

The water temperature in the experiment was 18 °C. The pH values fluctuated between 7.2 and 7.8 and for total hardness between 300 and 400 mg l/CaCO₃. The dissolved oxygen dropped from the highest value of 8,8 mg/l O₂ to the lowest value of 4,7 mg l/O₂. The titer of the saprophytic bacteria ascended steadily from 10² to 10⁶.

The phenol concentrations decreased in all tubes by ca. 20 % during the beginning of the experiment, while at the end they were even lower than 60 %. Physico-chemical parameters, measured in the control-set, were similar to those in the experimental sets, except that the control-set had less bacteria and more oxygen. No phenol was found in the water and in the animals.

With the *A. puella* species we noted that often, at the same time, in almost the same phenol concentrations very different accumulation values were found (Tab. 1). In the

Table 1. Phenol accumulation per gramme of bodyweight in the alive and dead individuals of the species *Agrion puella* depending on time, death rate and phenol concentration in the water

Tabelle 1. Phenolakkumulation je Gramm Körpergewicht in den lebenden und toten Individuen von *Agrion puella* in Abhängigkeit von der Zeit, der Mortalitätsrate und der Phenolkonzentration im Wasser

Time of exposure	Phenol concentration mg/l	Death rate %	Phenol accumulation	
			µg/g alive	dead
1. day	50	0	555,1	—
	75	0	85,95	—
	100	0	98,3	—
	125	0	25,14	—
	150	0	211,6	—
	175	20	233,63	155,03
	200	20	286,2	154,83
	Control	0	0	
2. day	50	0	162,19	—
	75	10	138,29	163,63
	100	10	148,51	202,7
	125	30	103,39	155,62
	150	20	106,97	63,24

Table 1 continued

Time of exposure	Phenol concentration mg/l	Death rate %	Phenol accumulation	
			$\mu\text{g/g}$ alive	dead
3. day	175	60	400,0	187,14
	200	100	—	46,59
	50	20	—	—
	75	20	150,34	21,02
	100	20	225,56	17,78
	125	60	20,91	17,0
	150	70	81,08	23,09
	175	90	309,73	16,19
4. day	200	100	—	—
	50	70	555,55	—
	75	70	92,10	—
	100	60	64,28	122,8
	125	100	—	134,54
	150	100	—	142,01
	175	100	—	—
5. day	200	100	—	—
	No dying established			
6. day	50	70	4,61	—
	75	50	12,55	38,51
	100	30	9,77	12,37
	125	100	—	—
	150	100	—	—
	175	100	—	—
	200	100	—	—
	Control	0	0	—

low concentrations of 50 mg/l the alive individual had accumulated 555 $\mu\text{g/g}$ of body-weight, while during the rather higher concentrations of 125 mg/l only 25 $\mu\text{g/g}$ was accumulated. Such anomalies were noted all the time, until the end of the experiment, more so in the alive than in the dead larvae.

The death-rate grows progressively in accordance with the increase of the concentration. On the first day a death-rate of 20 % individuals was noted — the phenol concentrations being 175 and 200 mg/l. On the 2nd d, LC_{100} was achieved already with the starting dose of 200 mg/l. On the fourth day LC_{100} was achieved in all concentrations of 125 mg/l and higher. LC_{50} was noted on the 6th d in the concentration of 75 mg/l.

There was no kill in the control-set.

Discussion and Conclusions

The *A. puella* larvae showed a great sensitivity to the added phenol. During the acute toxicity conditions this species absorbs great doses of phenol inside their organism, which are eliminated quickly. Therefore, we have very different results of

the accumulation, with a very rapid in- or decrease. Without regard to such a great fluctuation, the damages caused by acute poisoning have turned shown to be lethal during chronic conditions. The chitinous cuticle of this species seems to be rather permeable when submitted to the temperature of the experiment. Especially sensitive is the cuticle on the gills since it has been noted that after the gills have been destroyed, the animal dies very quickly.

The state of the metabolism as well as the elimination rate of phenol are specific in this case. The larvae accumulate during the first day of the experiment, rather great quantities of phenol, which is very quickly eliminated. After that they take again rather high doses of phenol which seems then to be lethal for this species which is killed rather quickly afterwards (Fig. 1).

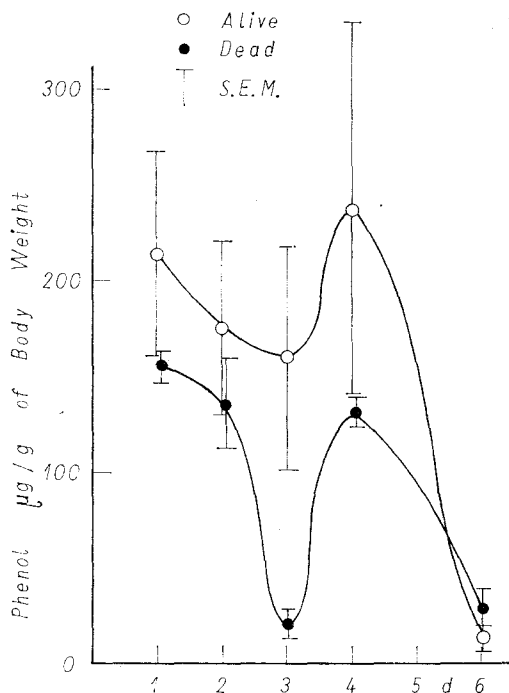


Fig. 1. Phenol accumulation in larvae of *Agrion puella*

Abb. 1. Phenolakkumulierung in Larven von *Agrion puella*

The dead, as well as the alive individuals, accumulate phenol equally, but the dead individuals show a much lower accumulation in — other words, a much smaller quantity is enough to be lethal.

The species *A. puella* often accumulates more phenol in lower concentrations, than under the influence of the higher one. This, taking into consideration the death-rate percentage, brings us to the conclusion that the same accumulation dose in various concentrations is not always equally lethal. This means that the measured values of the accumulated phenol are not always equally adequate, they depend upon the state of the metabolism and, connected with it, the intake and elimination rates of phenol. In higher concentrations, however, the accumulation as well as the death-rate increase. This is especially emphasized in the chronic poisoning conditions, when these enlarged concentrations which originate from the beginning of the experiment cause

the sudden death of the animals. Namely, the higher the accumulation, the shorter is the coma-period of the animals. In lower concentrations, the comas are longer. At the end of the experiment, in any case, the phenol accumulation falls to very low values in both, the dead and alive individuals. The lower lethal accumulation limit of chronic poisoning is near to 20 $\mu\text{g/g}$ of bodyweight, while in the acute poisoning conditions this limit is impossible to establish. The diminished accumulation is probably caused by the decreasing of the phenol concentration towards the end of the experiment, which is probably due to some of the saprophytic bacteria which use phenol as a source of carbon, on the other hand a small part of phenol evaporates. Under these circumstances the animals eliminate more phenol than taken into their bodies. However, since the organism is already exhausted and the metabolism is completely damaged, the animals die after a short period.

The loss of the dissolved oxygen concentration brought a higher death-rate upon the animals in the experiment without aeration. After that aeration was brought in.

The pH values as well as those of the total hardness did not contribute to an increase or decrease of phenol toxicity, although some authors (HERBERT; PICKERING and HENDERSON; KAWATSKI et al.) believe that the change of the pH as well as the increase of total hardness lessen the toxicity of this compound.

References

- APHA (1967): Standard Methods for the Examination of Water and Wastewater, 12th ed. New York, Amer. Publ. Health Assoc., 1967.
- ALEKSEEV, B. A., and N. E. USPENSKAJA: Toksikologiĉeskaja karakteristika ostrogo fenoljnovo otravljenjija njeikatorih prjesnovodnih ĉervjeĉ. (Toxicological characteristic of acute phenol intoxication in some freshwater worms). *Gidrobiologiĉeskij žurnal*, **10** (1974), 4, 48–55.
- BACH, A.: Phenolschwund in Wasser. — *Ges. Ing.* **52** (1929), (cit. H. LIEBMANN, 1960).
- ERBEN, R., V. JOVANOVIĆ, I. MUNJKO and L. ŠTILINOVIĆ: Utjecaj akumulacije fenola na preživljavanje puževa prednoškržnjaka (Prosobranchia, Gastropoda) iz voda na kopnu. (Influence of phenol accumulation upon the survival of snails from the freshwater). *Polj. i. šum.* **XXVIII** (1982), 1, in print, Titograd.
- HERBERT, D. W. M.: The toxicity to rainbow trout of spent still liquors from the distillation of coal. *Ann. Appl. Bio.* **50** (1962), 755–777.
- LIEBMANN, H.: *Handbuch der Frischwasser- und Abwasserbiologie*, Bd. 2. Jena, VEB Gustav Fischer Verlag, 1960, 933–943.
- KAWATSKI, J. A., V. K. DAWSON and M. L. RENVERS: Effect of TFM and Bayer 73 on in vivo oxygen consumption of the aquatic midge *Chironomus tentans*. *Trans. Amer. Fish. Soc.* **3** (1974), 551–556.
- PICKERING, P. H., and C. HENDERSON: Acute toxicity of some important petrochemicals to fish. *J. Water Pollut. Control Fed.* **38** (1966), 1419–29.

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