

The usage of *Daphnia magna* as alternative bioobject in ecotoxicology

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Abstract

We have been developing non-traditional methods of the identification of pollutants, using various hydrobionts as biological objects and the study of the mechanism of toxic action of xenobiotics. The experiments were carried out with using of *Daphnia magna*. *Daphnia magna* is a Crustacean in the order of Cladocera. Experiments were performed with a 2-days old culture of *Daphnia magna*. The toxicity of xenobiotics was determined by the value of LC_{50} , a concentration of the compounds causing death to 50% of hydrobionts during incubation with toxicants for 24 hours. In the first stage of the work, toxicity of organophosphates (Dipterex, DFP, DDVP, Paraoxon, Malathion, Malaoxon), heavy metals ions (Hg, Pb, Cu, Co, Cd, Cr, As, Al), organochlorines (Aldrin, Dieldrin, Endrin, Aroclor, DDT, Lindane, PCBs etc.), cyanides (sodium cyanide) and pyrethroids (Cypermethrin, Fenvalerate, Deltamethrin, Permethrin, Allethrin, Resmethrin, Phenothrin, Kadethrin, Cyphenothrin) was determined. The effects of a number of antagonists on the toxicity of xenobiotics were studied. At the first time we discovered that in experiments to *Daphnia magna* some muscarinic cholinoreceptor blockers (atropine, amyzil etc.) reduced a toxic the effect of organophosphates. In the case of heavy metals the chelating agents (EDTA, Dithioethylcarbamate, Unithiolum, Sodium thiosulphuricum, L-Aspartic acid) were effective, for certain organochlorine poisonings - anticonvulsive drugs (diazepam, phenobarbital), for cyanide poisoning sodium nitrite and anticyane. In the case of pyrethroid's poisonings the antagonist of glutamate receptor (ketamine) and agonists of GABA-receptor (phenazepam, ethanol) reduced the toxicity of xenobiotics. As far as these antidotes have a specific treatment action only against definite classes of pollutants, we have elaborated the sensitive express-methods of bioidentification of pollutants.

Keywords: *Daphnia magna*, alternatives, xenobiotics, bioidentification

Introduction

With a constant growth of the anthropogenic pressure on water bodies the development and usage of bioindication methods supplementing physical and chemical methods of xenobiotic identification acquires especial significance. In view of the fact that chemical analysis require special equipments, they are expensive to perform and do not allow to evaluate the environmental toxicity, during the recent decade large scale investigations have been performed to study various test-objects that are suitable for bioassay. At present biotesting plays an important role in the system of water quality control. On the currently used methods of bioassay provide only the integral evaluation of the pollutants effect but not the determination of the xenobiotics a origin (Flerov, 1989). We have been developing non traditional method for determination of different classes of pollutants using various hydrobionts as biological

test-objects and our knowledges of the mechanism of toxic action of xenobiotics. Knowing the mechanisms of the specific toxic action of poisons, it is possible to use various pharmacological compounds to decrease or increase the effects of toxicants. This approach allows us to use biological objects to identify certain xenobiotics, poisoning from which can be prevented by means of poisoning's antagonists. All above mentioned methods are widely used when employing experimental animals (mice, rats) as test-objects, but it has not been developed at all for alternative biological objects, particularly for hydrobionts. The elaboration of a new methods of bioidentification was founded on the study of Cholin-, GABA-, Dopamin- and Glutamate- ergic system of *Daphnia magna* and usage of pharmacological antagonists of xenobiotics. Such new pharmacological approach with usage of *Daphnia magna* as bioobject have made possible to perform the general identification of different

classes of the most toxic for aquatic ecosystem health xenobiotics (organophosphates, carbamates, heavy metals, organochlorines, pyrethroids) without usage of chemical analysis.

Materials and methods

As a background of discussed method, we have chosen a big amount of anticholinesterase (antiChe) compounds, different heavy metals, organochlorine pesticides and pyrethroids. Many compounds of these classes are used as pesticides, drugs and chemical warfare agents. Currently, dozens of pesticides capable of polluting the aqueous media through the runoff from agricultural lands or as a result of chemical industry accidents are produced. The experiments were carried out with using of *Daphnia magna*. *Daphnia magna* is a Crustacean in the order of Cladocera. This aquatic animal extensively used as a test organism in aquatic toxicology due to their small size, short life cycle and amenability to lab culture. *Daphnia magna* is the most sensitive test-object in relation of different pollutants (organophosphates, heavy metals, organochlorines, pyrethroids etc.) among all known biological objects including experimental animals (Peters and De Bernardi, 1987). Experiments were performed with a 2-days old culture of *Daphnia*

Table 1. The influence of cholinolytics on toxicity of DDVP and aminostigmine in experiments to *Daphnia magna*.

Drugs mg/l	LC ₅₀ DDVP, mg/l	PC	LC ₅₀ aminostigmine, mg/l	PC
Control	0.00021±0.00005	-	0.012±0.002	-
Atropine				
1.0	0.00052±0.00004	2.5	0.018±0.002	1.5
2.0	0.00063±0.00007	3.0	0.042±0.009	3.5
6.0	0.00073±0.00006	3.5	0.042±0.009	3.5
Glipine				
1.0	0.00042±0.00008	2.0	0.021±0.005	1.75
2.0	0.00074±0.0001	3.5	0.069±0.019	5.75
Pediphen				
1.0	0.00032±0.00008	1.5	0.024±0.002	2.0
2.0	0.00063±0.00008	3.0	0.036±0.004	3.0

magna. During the experiments, hydrobionts were placed in beakers with 25 ml of dechlorinated settled tap water at 18-20°C. The toxicity of xenobiotics was determined by the value of LC₅₀, a concentration of the compounds causing death to 50% of hydrobionts during incubation with toxicants for 24 hours. In the first stage of the work, toxicity of organophosphates and carbamates (Dipterex, DFP, DDVP, Paraoxon, Malathion, Malaaxon, Aminostigmine, Physostigmine, Sevine), heavy metals (Hg, Pb, Cu, Co, Cd, Cr, As, Al), organochlorines (Aldrin, Dieldrin, Endrin, Aroclor, DDT, Lindane, PCBs etc.) and pyrethroids (Cypermethrin, Fenvalerate, Deltamethrin, Permethrin, Allethrin, Resmethrin, Phenothrin, Kadethrin, Cyphenothrin) was determined. The effects of a number of poisons antagonists on the toxicity of xenobiotics were studied. Xenobiotics and their antagonists were added to the incubation mixture simultaneously. The results of the protection experiments are expressed as the protective coefficient (PC) – the ratio of LC₅₀ value in treated and in untreated *daphnids*.

Results

On the base of study of mechanism of xenobiotics action to *Daphnia magna* and the usage of pharmacological antagonists of poisonings the new methods of bioidentification of different pollutants were elaborated. At the first time we discovered that in experiments to *Daphnia magna* some muscarinic cholinoreceptor blockers (atropine, glipine, pediphen etc.) reduced a toxic the effect of organophosphates and carbamates (Table 1). In the case of heavy metals the chelating agents (EDTA, Dithioethylcarbamate, Unithiolum, Sodium thiosulphuric, L-Aspartic acid) were effective (Table 2), for certain organochlorine poisonings - anticonvulsive drugs phenazepam, phenobarbital (Table 3). In the case of pyrethroid's poisonings the antagonists of glutamate (ketamine), DOPA (haloperidole) receptors and blockers of calcium channel (nimodipine) reduced the toxicity of xenobiotics (Table 4, 5). As far as these antidotes have a specific treatment action only against definite classes of pollutants, we have elaborated

Table 2. The influence of GABA-mimetics on toxicity of DDT and lindane in experiments to *Daphnia magna*.

Compounds mg/l	LC ₅₀ mg/l DDT	PC	LC ₅₀ mg/l Lindane	PC
Control	0.08±0.02	-	0.12±0.02	-
Ethyl alcohol (g/l)				
0.1	0.15±0.03	1.9	0.23±0.07	1.9
0.025	0.24±0.03	3.0	0.26±0.07	2.2
Phenobarbital				
2.0	0.21±0.03	2.6	0.24±0.06	2.0
1.0	0.23±0.07	2.9	0.32±0.09	2.7
Phenazepam				
0.1	0.32±0.09	4.0	0.28±0.09	2.3
0.05	0.25±0.07	3.1	0.23±0.08	1.9

Table 3. The influence of EDTA and Unithiolum on toxicity of Pb(NO₃)₂ and HgCl₂ in experiments to *Daphnia magna*.

Chelates (mg/l)	HgCl ₂ LC ₅₀ (mg/l)	PC	Pb(NO ₃) ₂ LC ₅₀ (mg/l)	PC
Control	0.16±0.06	-	1.70±0.52	-
EDTA				
2.5	1.59±0.47	9.97	3.38±0.56	1.98
5.0	2.1±0.05	13.1	5.1±0.56	3.0
10.0	3.2±0.06	20.0	6.6±1.48	3.88
25.0	-	-	13.3±2.9	7.8
Unithiolum				
25.0	0.46±0.11	2.87	3.38±0.56	1.98
50.0	1.38±0.38	8.60	7.67±1.79	4.5
100.0	1.69±0.28	10.6	13.45±2.24	7.9

Table 4: The influence of ketamine and nimodipine on the toxicity of pyrethroids in experiments to *Daphnia magna*.

Pyrethroids (mg/l)	Cypermethrin LC ₅₀ (mg/l)	PC	Phenothrin LC ₅₀ (mg/l)	PC
Control	0.057±0.003	-	0.05±0.12	-
Ketamine				
7.5	0.62±0.17	10.9	0.52±0.12	10.4
4.0	0.51±0.13	8.9	0.46±0.10	9.2
Nimodipine				
25.0	0.29±0.03	5.1	0.42±0.03	8.4
12.5	0.16±0.02	2.8	0.21±0.09	4.2

Table 5. The influence of haloperidole on the toxicity of pyrethroids to *Daphnia magna*

Pyrethroids LC ₅₀ (mg/l)	Cypermethrin LC50 (mg/l)	PC	Phenothrin LC ₅₀ (mg/l)	PC	Permethrin LC ₅₀ (mg/l)	PC
Control	0.057	-	0.05	-	0.05	-
Haloperidole						
3.0	0.46	8.1	0.6	12.0	0.36	7.2
1.5	0.48	8.4	0.55	11.6	0.50	10.0

the sensitive express-methods of bioidentification of pollutants. Such new pharmacological approach with use of hydrobionts as test-objects have made possible to perform the general identification of different classes of xenobiotics in fresh water.

Conclusions

We have been developing non-traditional express-method of the identification of pollutants/ organophosphates, carbamates, organochlorines, heavy metals and pyrethroids/ using *Daphnia magna* as biological object and the study of the mechanism of toxic action of xenobiotics. The new method was proposed for water pollution control.

References

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