Journal of Environmental Protection, 2013, 4, 585-594
http://dx.doi.org/10.4236/jep.2013.46068 Published Online June 2013 (http://www.scirp.org/journal/jep)

Complexation with ATP as a Cause of Pollutants Toxicity to Aquatic Life

Elena A. Saratovskikh

Institute of Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Russia.
Email: easar@icp.ac.ru

Received March 31st, 2013; revised May 2nd, 2013; accepted June 1st, 2013

ABSTRACT

The toxicity of some pesticides, namely, Lontrel, Sencor, Roundup, Bentazon, and Hymexazol, and also Lontrel complexes with Cu, Co, Mn, Mg, Mo, Ni, and Zn that are being widely used in agriculture and, hence, abundant in the environment were characterized by biotesting. The variation in the enzymatic activity of the luminous bacteria *Beneckea harveyi* and in the reproductive function of the infusoria *Tetrahymena pyriformis* was measured and used to determine toxicity factors. The toxicity of the above compounds was found to correlate with their ability to form stable complexes with adenosine triphosphate (ATP).

Keywords: Pesticides; Lontrel Complexes with Metals; Biotesting; Toxicity; Correlation with Complexes of ATP

1. Introduction

Nowadays, different kinds of chemicals originating from the agricultural use of pesticides eventually become deposited in soil, water basins, and ground water. Ever increasing human impact to the nature puts forward an acute problem of environment protection [1-3]. Proper nature-conservative measures can be suggested only on the basis of deep insight into the mechanism of effect of toxic compounds at the cellular and sub-cellular level. We have to know a relationship between the mechanism of the toxicant action and the effect produced by a pollutant (Plt) on the entire organism. Most adequately, such a relationship can be established by the methods of biotesting using either entire test-objects or their functional fragments.

Adenosine triphosphate (ATP) is the only biomolecule that supports the processes of energy storage/release in living organisms. Previously, we explored the effect of Plt on biological objects on the molecular level: the complexation of technogenic toxicants with ATP mononucleotide, its structural analog (ε-ATP), metals, and di/polynucleotides [4-7]. Binding of Plt with ATP gives rise to energy shortage in cells, which may block the functioning of tricarboxylic acids cycle, interrupt electron transfer processes, and, as a result, suppress photosynthetic processes.

In this work, we investigated the toxicity of some pesticides, viz., Lontrel, Sencor, Roundup, Bentazon, and Hymexazol, and also Lontrel complexes with Cu, Co, Mn, Mg, Mo, Ni, and Zn by the methods of biotesting. We aimed at elucidating a relationship between the toxicity of the above compounds and their ability to form stable complexes with ATP.

2. Material and Methods

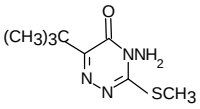
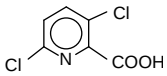
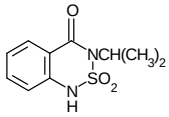
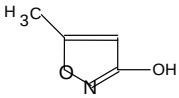
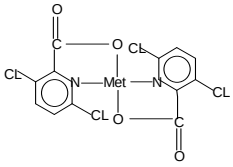
2.1. Used Substances, Concentrations and Replicates

In experiments, we used the pesticides characterized in **Table 1**. Their isolation from commercially available chemicals and purification were carried out as described elsewhere [4]. The Lontrel complexes with metals were synthesized as reported previously [8,9].

ε-ATP was synthesized as proposed by [4]. Equilibrium constants K_c for the complexation of Plt with ATP and ε-ATP were estimated from the fluorescence quenching curves using a mathematical model suggested by us previously [4]. Fluorescence measurements were performed with an Aminco-Bowman spectrofluorimeter. The excitation of ATP at λ_{exc} = 290 nm gave rise to a fluorescence band with a maximum at λ_{max} = 380 nm; for ε-ATP, λ_{exc} = 312 nm and λ_{max} = 420 nm.

Toxicity of the pesticides under study was detected over a wide range of their concentration, from 10^{-3} up to 10^{-1} M, in view of the fact that the concentration rec-

Table 1. Commercial names and chemical formulae of the studied pesticides.

Commercial name	Chemical class	Chemical name	Chemical formula
Sencor, Metribuzin	Triazines	4-amino-6-*tert*-butyl-3-methylthio-1,2,4-triazinone-5	
Lontrel, Clopyralid	Pyridines	3,6-dichloropicolinic acid	
Glyphosate, Roundup, Rodeo	Phosphorus-containing	*N*-phosphone-methylglycine	$(OH)_2POCH_2NHCH_2COOH$
Bentazon Basagran	Sulfonamides	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide	
Hymexazol Tachigaren	Isoxazoles	3-hydroxy-5-methylisoxazole	
	Lontrel-metal complexes (ML_2)	bis-(3,6-dichloropicolinato)metal(II)	

ommended for agricultural use is known to have a value of 10^{-2} M. Stronger toxicity of Lontrel complexes with metals was explored within the concentration range 10^{-2} - 10^{-8} M.

2.2. Toxicological Testings

2.2.1. The Biotest on *Beneckea harveyi*

In experiments, we monitored a change in the enzymatic activity of the luminous bacteria *Beneckea harveyi*, which becomes noticeable already in 30 min after incubation [3,10,11].

NaCl (reagent grade, from Agat-Med, Russia) was additionally purified by double recrystallization. Water was used after bidistillation. The culture of luminous marine bacteria *Beneckea harveyi*, viz., Photobacterium *phosphoreum*, was prepared at the Institute of Biophysics (Siberian Branch, Russian Academy of Sciences, Krasnoyarsk).

For experiments, lyophilized cells of the bacteria *B. harveyi* (normally kept in a deep-freezer) were suspended in a 3% aqueous solution of NaCl. To 0.5 ml of such a solution, 0.3 - 0.5 ml of suspended bacteria was added. A 0.85% solution of NaCl was used as a reference sample. The luminescence intensity I of the enzyme luciferase present in the cells (λ_{exc} = 290 nm, λ_{max} = 340 nm) was monitored with a BLM-8801 luminometer. A decrease in I (compared to that of the reference sample) measured in the presence of added pesticide was regarded as a measure of toxicity T for a given compound [3,10,11].

A decrease in I can be associated either with the inhibition of luciferase or with the effect of the toxicants on some other units of the metabolism chain. The toxicity index T was determined from the expression

$$T = \left[\left(I_r - I_{mes}\right)/I_r\right] \times 100\%, \quad (1)$$

where I_r is the luminescence intensity of the reference sample, and I_{mes} is the value measured for a solution containing a given pesticide. For $T \le 19\%$, the pesticide is regarded as nontoxic. For $19 < T \le 50\%$, the pesticide is considered as toxic, while for $T > 50\%$, it is regarded as strongly toxic.

2.2.2. The Biotest on *Tetrahymena pyriformis*

In another set of experiments, we measured the effect of pesticides on the reproductive function of the infusoria *Tetrahymena pyriformis* upon a 24-h exposure as described in [10,11]. Similarly, the toxicity factor K was calculated using the expression

$$K = \left[\left(A_r - A_{mes}\right)/A_r\right] \times 100\%, \quad (2)$$

where A_r is an increment in the total number of infusoria cells in the reference sample, and A_{mes} is that measured for the sample containing a given pesticide. A pesticide was regarded as toxic when $K > 50\%$. The reliability of the data was estimated using the Student criterion.

In both cases, the EC_{50} values, toxicant concentrations ensuring a 50% effect, were determined graphically using the procedure described in [10].

3. Results and Discussion

3.1. Toxicity of Pesticides with Respect to Luminous Bacteria and Infusoria

Toxicity factors T towards the luminous bacteria *B. harveyi* as a function of pesticide concentration C are shown in **Figure 1**. It follows that K increases proportionally to C, although the slopes of the linear plots differ: the largest slope is observed for Sencor and the smallest one is observed for Hymexazol. Even at $C = 10^{-1}$ M Hymexazol remains nontoxic ($T < 19\%$). Bentazon is seen to be lowly toxic over the whole range of C ($24\% < T \leq 40\%$). In the range from 10^{-3} to 3×10^{-3} M, all of the compounds under study are lowly toxic ($T \leq 50\%$). Sencor becomes highly toxic at $C = 5 \times 10^{-3}$ M, Lontrel becomes highly at 10^{-2} M, while Roundup is highly toxic at 10^{-1} M.

Toxicity factors K towards the infusoria *T. pyriformis* vs. C are presented in **Figure 2**. Again, the plots are seen to be linear, the toxicity of Sencor is the largest, while that of Hymezazol is the lowest. Even at $C = 10^{-3}$ M all of the compounds under consideration are highly toxic ($K > 50\%$). The slope of the linear plots is seen to decrease in the order Sencor > Lontrel > Roundup > Bentazon > Hymexazol. With increasing C, the toxicity of Hymexazol increases more slowly than that of Sencor by a factor of five.

Our data on the EC_{50} values are collected in **Table 2**. It follows that these are markedly close in their magnitude and increase in the order Sencor < Lontrel < Roundup < Bentazon < Hymexazol from 2.63×10^{-3} M (0.56 g/l) to 9×10^{-1} M. Generally, the EC_{50} values measured for *B. harveyi* are somewhat lower than those for *T. pyriformis*.

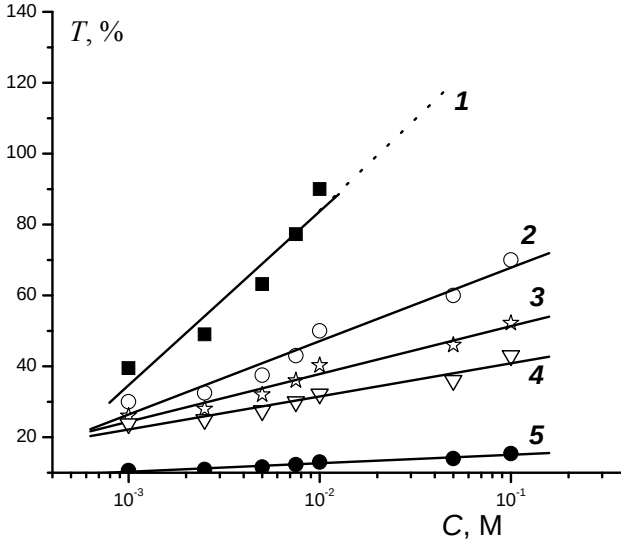


Figure 1. Dependence of the toxicity of pesticides with respect to luminescent bacteria on pesticide concentrations: 1—Sencor; 2—Lontrel; 3—Glyphosate; 4—Bentazon; 5—Hymexazol.

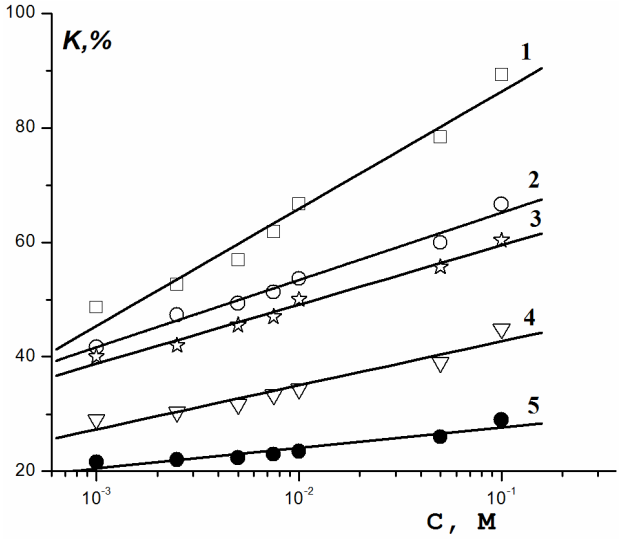


Figure 2. Dependence of the toxicity of pesticides with respect to infusoria on pesticide concentrations: 1—Sencor; 2—Lontrel; 3—Glyphosate; 4—Bentazon; 5—Hymexazol.

Table 2. Values of EC_{50} measured for pesticides by the biotesting method with the use of fluorescent bacteria *Beneckea harveyi* and infusoria *Tetrahymena pyriformis*.

Pesticide	$K_c \times 10^{-3}$, M^{-1} [4]	EC_{50}			
		Beneckea harveyi		*Tetrahymena pyriformis*	
		M	g/l	M	g/l
Sencor	26.5 ± 3.3	4.4×10^{-3}	0.94	2.63×10^{-3}	0.56
Lontrel	15.9 ± 2.0	5.0×10^{-3}	0.96	3.24×10^{-3}	0.62
Roundup	8.2 ± 1.2	9.1×10^{-3}	1.54	1.32×10^{-2}	2.23
Basagran	4.7 ± 0.4	2.9×10^{-2}	7.01	5.0×10^{-2}	12.0
Tachigaren	1.1 ± 0.04	8.9×10^{-1}	88.20	9.0×10^{-1}	89.20
Tilt	0.8 ± 0.06	"-"			
Setoxidim	5.0 ± 0.3	"-"			
Kusagard	9.7 ± 0.5	"-"			

3.2. Determination of Toxicity for the Metal Complexes

As known [8,9], some Plt are capable of forming various complexes that stable in soil and aquatic environment even under the action of microorganisms and UV irradiation. In addition, these complexes were found [3] to exhibit high reactivity with respect to biomolecules. However, any quantitative data on the toxicity of such Plt-metal complexes are still lacking in the literature. In this work, we explored the toxicity of the $MetL_2$ complexes towards the bacteria *B. harveyi*, where ligand L is Lontrel (3,6-dichloropicalinic acid) and Met = Cu, Co, Mn, and Mg. As follows from **Figure 3**, these complexes exhibit toxicity even at $C = 10^{-7}$ M. Since curves 1 - 4 are parallel,

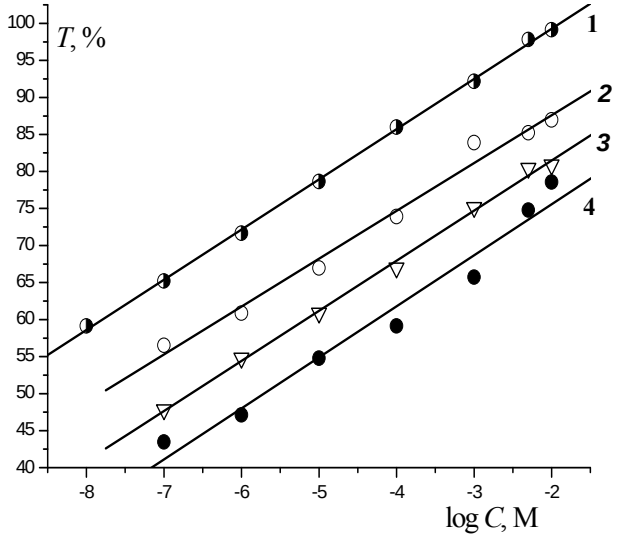


Figure 3. Dependence of the toxicity of the Lontrel-metal complexes with respect to luminescent bacteria on the logarithm of the concentration of complexes: 1—MnL_2; 2—CuL_2; 3—CoL_2.

for different complexes T increases with increasing C with an identical rate. For all C, the values of T decrease in the order $CuL_2 > CoL_2 > MnL_2 > MgL_2$, as well as the EC_{50} values (**Table 3**). Note that the toxicity of Lontrel complexes turned out to be higher than that of Lontrel itself. Compared to Lontrel, the EC_{50} value for MgL_2 is lower by a factor of two, while that for CuL_2 is lower by three orders of magnitude.

As seen in **Figure 4**, CuL_2, CoL_2, NiL_2, and MoL_2 ($C = 10^{-5}$ M) also exhibit toxicity towards the infusoria *T. pyriformis* ($K > 50\%$). For $C < 10^{-5}$ M, MnL_2, ZnL_2, and MgL_2 are nontoxic ($K < 50\%$). The above complexes are always toxic for $C > 10^{-4}$ M. Again, curves 1 - 7 are nearly parallel. At any C, the K values decrease in the order $CuL_2 > CoL_2 > NiL_2 > MoL_2 > MnL_2 > ZnL_2 > MgL_2$. MgL_2 was the only complex that exhibited a lower toxicity compared to that of Lontrel (at any C). The K values for CuL_2, CoL_2, and NiL_2 were higher than that for Lontrel by a factor of two, while those for MoL_2, MnL_2, and ZnL_2 were higher by a factor of 1.5.

As follows from **Table 3**, the EC_{50} value for MnL_2 is lower than that for Lontrel by a factor of five. Those for NiL_2, MoL_2, and CoL_2 are lower by a factor of ten, where that for CuL_2 is lower by a factor of 16. For the infusoria *T. pyriformis*, the respective EC_{50} values turned out to be higher that those for *B. harveyi* by 1 - 2 orders of magnitude. Tentatively, this can be associated with the fact that in experiments *in vitro* with *B. harveyi* we are dealing with the deactivation of a sole enzyme (luciferase) by contrast to *in vivo* experiments with *T. pyriformis*.

A living organism is known to synthesize metallothioneines, low-molecular-weight cysteine-rich proteins,

Table 3. Values of EC_{50} measured for Lontrel-metal complexes by the biotesting method with the use of fluorescent bacteria *Beneckea harveyi* and infusoria *Tetrahymena pyriformis*.

Complex	$K_c \times 10^{-3}$, M^{-1} [4]	EC_{50}			
		Beneckea harveyi		*Tetrahymena pyriformis*	
		M	g/l	M	g/l
CuL_2	851.4 ± 82	5.6×10^{-6}	0.0025	2.0×10^{-4}	8.9×10^{-2}
CoL_2	600 ± 200	6.3×10^{-5}	0.0278	3.0×10^{-4}	1.33×10^{-1}
NiL_2	21.6 ± 0.5	"-"	"-"	3.2×10^{-4}	1.43×10^{-1}
MoL_2	3.6 ± 0.4	"-"	"-"	4.0×10^{-4}	1.9×10^{-1}
MnL_2	2.2 ± 0.1	2.0×10^{-4}	0.087	6.3×10^{-4}	2.76×10^{-1}
ZnL_2	1.6 ± 0.06	"-"	"-"	1.0×10^{-3}	4.5×10^{-1}
MgL_2	0.8 ± 0.02	1.0×10^{-4}	0.041	1.2×10^{-3}	4.7×10^{-1}
Lontrel	15.9 ± 2	5.0×10^{-3}	0.960	3.24×10^{-3}	0.62

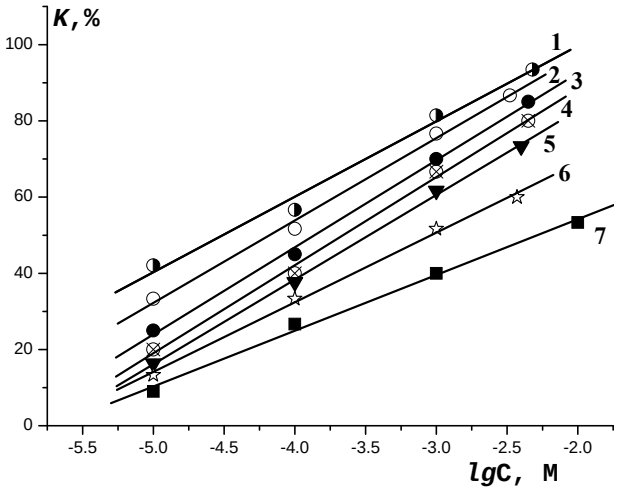


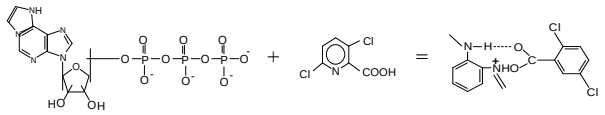
Figure 4. Dependence of the toxicity of the Lontrel-metal complexes with respect to infusoria on the logarithm of the concentration of complexes: 1—MnL_2; 2—CuL_2; 3—CoL_2.

designed for binding metal cations [12] and some organic xenobiotics, such as pesticides [13] for their subsequent elimination from the organism. Such a self-protecion system is present in all living organisms, including representatives of aquatic life. Probably, this can explain the higher resistivity of the cells in *T. pyriformis* to the action of metal complexes with Lontrel.

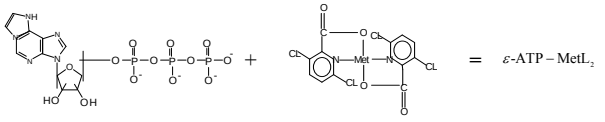
As follows from **Tables 2** and **3**, the metal complexes become toxic in concentrations C two orders of magnitude lower than those of free pesticides. Accordingly, the toxicity of $MetL_2$ complexes is two orders of magnitude higher than that of Lontrel.

As known [4], some pollutants (L) are capable of forming complexes with nucleotides, in particular, with ATP and its structural analog ε-ATP

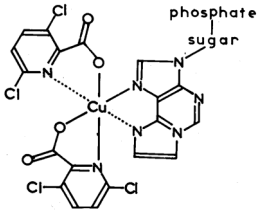
$$\varepsilon\text{-ATP} - \text{L} \leftarrow \frac{k^{+1}}{k^{-1}} \rightarrow = \varepsilon\text{-ATP} - \text{L}$$



Ternary complexes of ATP with metals are formed similarly



The structure of the ε-ATP—CuL_2 complex [4] is illustrated below



3.3. Correlation between the Energy Shortage and Toxicity

Since energy metabolism is one of the key processes taking place in living organisms and ATP is the only biomolecule responsible for assimilation and dissimilation of energy, the above complexation can be expected to infringe energy metabolism and, hence, lead to energy shortage in cells [7]. In turn, the energy shortage blocks the functioning of the tricarboxylic acid cycle, electron transport, and photosynthetic processes. As a result, this was found to cause the death of plant organisms, as it has been demonstrated for pea and wheat crops [6].

Similar correlation between the complex formation with ATP leading to energy shortage in cells and the toxicity to the soil-living microarthropods *Xenylla grisea (Hypogastruridae)* and *Folsomia candida* (*Isotomidae)* was established by [14]. In this context, it seemed interesting to correlate the energy shortage in cells of aquatic organisms caused by a given pesticide to the equilibrium constant K_c for complexation with ATP. Therefore, the values of K_c given in **Tables 2** and **3** will be used to characterize the above energy shortage in cells.

In **Figure 5**, the toxicity T towards the bacteria *B. harveyi* as a function of K_c for complexation with ε-ATP at various C (from 10^{-1} to 10^{-3} M) is seen to be directly proportional.

The toxicity K towards the cells of *T. pyriformis* as a function of K_c is shown in **Figure 6**. Again, the K values

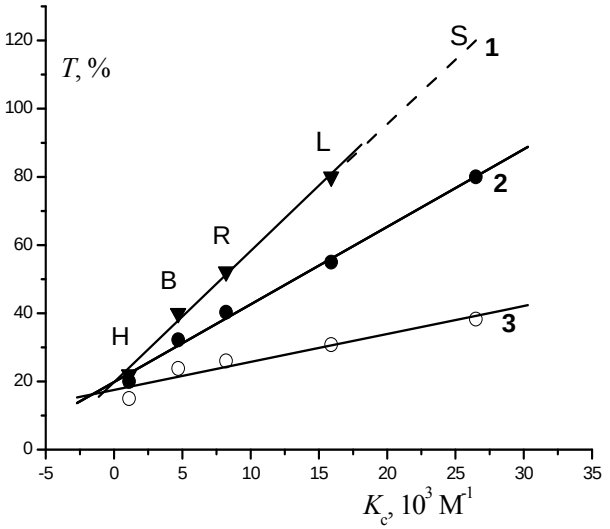


Figure 5. Correlation of the toxicity of pesticides with respect to luminescent bacteria with the values of constant of [ε-ATP-pesticide] complex formation (K_c) for pesticide concentrations: 1—10^{-1} M; 2—10^{-2} M; 3—10^{-3} M. S—Sencor; L—Lontrel; B—Bentazon; R—Glyphosate; H—Hymexazol.

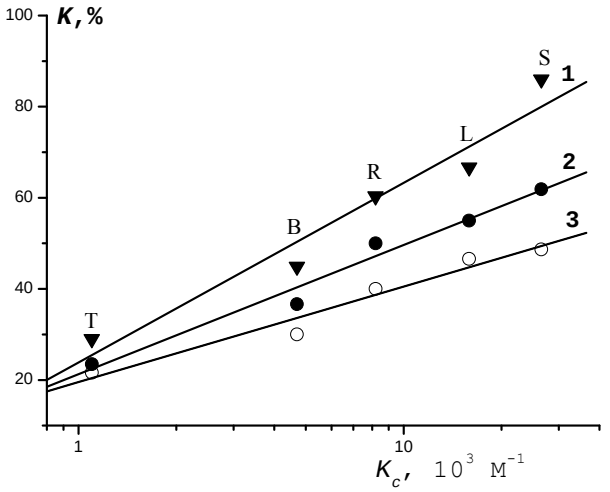


Figure 6. Correlation of the toxicity of pesticides with respect to infusoria with the values of constant of [ε-ATP-pesticide] complex formation (K_c) for pesticide concentrations: 1—10^{-1} M; 2—10^{-2} M; 3—10^{-3} M; S—Sencor; L—Lontrel; B—Bentazon; R—Glyphosate; H—Hymexazol.

are seen to be proportional to K_c over the entire range of C (from 10^{-1} to 10^{-3} M).

The toxicity of metal complexes towards *B. harveyi* and *T. pyriformis* as a function of K_c are shown in **Figures 7** and **8**, respectively, as a series of parallel linear plots. With increasing K_c, the toxicity of the complexes under study is seen to increase proportionally over the entire range of C (10^{-1} - 10^{-7} M).

The EC_{50} values as a function of K_c for our pesticides are given in **Figure 9**. Using this plot, we attempted to predict the EC_{50} values based on a known magnitude of

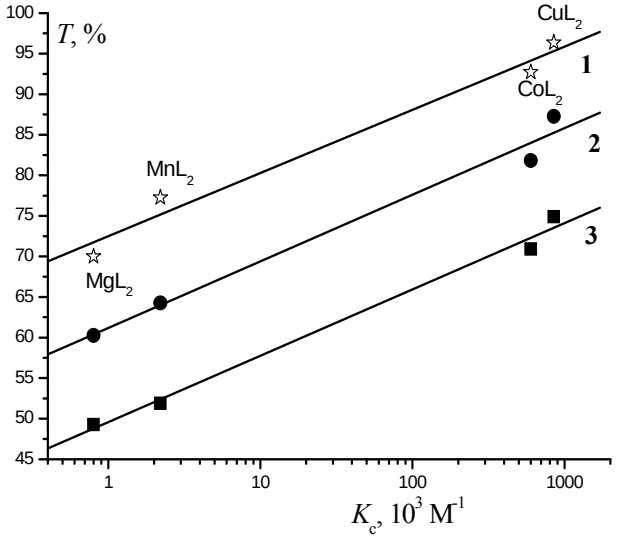


Figure 7. Correlation of the toxicity of Lontrel-metal complexes with respect to luminescent bacteria with the values of constant of [ε-ATP-MetL$_2$] complex formation (K_c) for Lontrel-metal complexes concentrations: 1—10^{-1} M; 2—10^{-2} M; 3—10^{-3} M.

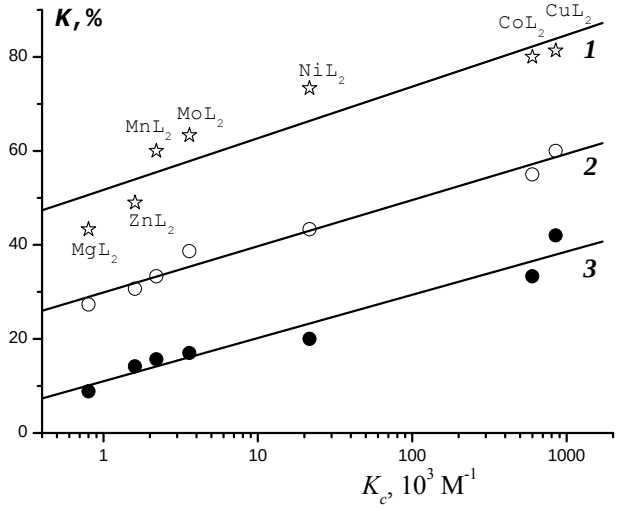


Figure 8. Correlation of the toxicity of Lontrel-metal complexes with respect to infusoria with the values of constant of [ε-ATP-MetL$_2$] complex formation (K_c) for Lontrel-metal complexes concentrations: 1—10^{-1} M; 2—10^{-2} M; 3—10^{-3} M.

K_c. For this purpose, we drew the reported values of K_c for Kuzagard, Setoxydime, and Tilt onto curve 1 in **Figure 9**. Our curve 1 predicts that for the above compounds the EC_{50} values must attain the values of 1.0×10^{-2}, 3.4×10^{-2}, and 1.1 M, respectively.

Just as for pesticides, we compared the behavior of the complexes at the same EC_{50} value and plotted the de pendence of this EC_{50} on K_c (**Figure 10**). Apparently, this plot holds true for the metal complexes with a given ligand (L). This dependence is universal: it relates the

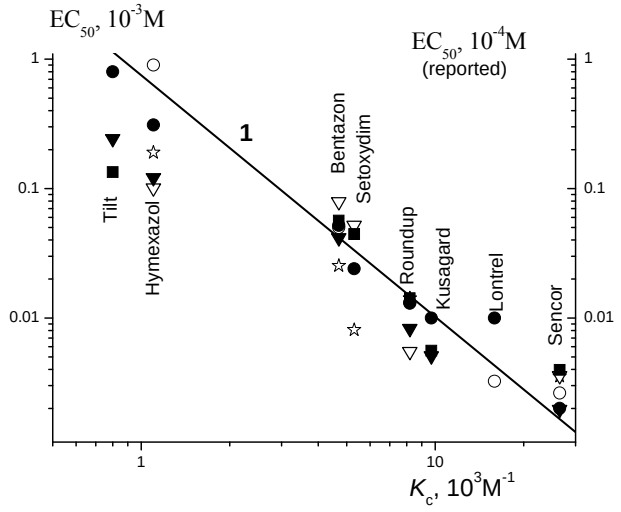


Figure 9. Regularity of the dependences of the values EC_{50} of pesticides with respect to infusoria on values of constant of complex formation (K_c): (○) our data points for the infusoria *Tetrahymena pyriformis* and the reported data for (●) zooplankton *Daphnia magna* [21]; (☆) juvenile fish *Lepomis macrochirus* up to 0.4 cm in size [21], (▽) juvenile fish *Oncorhynchus mykiss* [21,34], (▼) adult fish *Oncorhynchus mykiss* [21,24,28-30], and (■) adult fish *Cyprinodon variegates* [21,22,28,32,33,35].

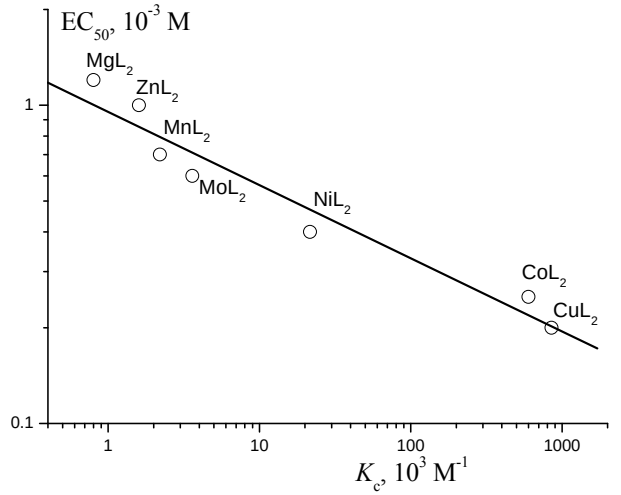


Figure 10. Regularity of the dependences of the toxicity of Lontrel-metal complexes with respect to infusoria on values of constant of complex formation (K_c).

energy shortage in cell caused by that or another Plt to the acute toxicity of a given Plt. and "principle".

3.4. Relationship between Available Experimental and Literature Data

It seemed interesting to check out the universality of the above relationship on the available set of relevant data (**Table 4**). For the water flea *Scapholeberis kingi*, the LC_{50} value for Roundup is seen to be lower than Benta-

zon. However, for the opossum *Americamysis bahia*, the LC_{50} values well correlate with K_c for the complexation of ATP with Roundup, Setoxydime, Bentazon, and Tilt.

Despite a wide use of Lontrel in agriculture, information about its toxicity is virtually lacking in the literature. The available data for the simplest ciliated *T. pyriformis* [15] and *T. thermophila* [16-18] indicate that the toxicity of Lontrel is higher than that of Hymexazol by a factor of 5 - 10 (EC_{50} = 104.6 × 10^{-6} g/l = 0.54 × 10^{-6} M and 500 × 10^{-6} g/l = 5.05 × 10^{-6} M, respectively). According to our data, this difference is only 2 - 2.5 times. Our data for the infusoria demonstrate the ability of the pesticides under study to complexation with ATP. The reported data for zooplankton *Daphnia magna* are summarized in **Figure 9**. Our curve 1 and the EC_{50} values reported by different workers for six pesticides are seen to correlate.

The reported LC_{50} values for *Crustacea* are collected in **Table 5**. The toxicity of Roundup (to *Orconectes nais*), Hymexazol (to *Decapoda*), and Tilt (to *Procambarus* sp.) are seen to decrease in the above order according to our K_c values for these compounds.

The LC_{50} values for different fishes are summarized in **Table 6** and **Figure 9**. As follows from **Figure 9**, our curve 1 well agrees with the available data for zooplankton and fish. These data also confirm a correlation between the Plt toxicity and the molecular mechanism of their action, namely, ATP binding in living cells.

As known, on the one hand, all living organisms, including fish, possess a unique system for excretion of foreign chemical compounds [12]. On the other hand, the pollutants under study influence not only on luciferase but also on the entire system of redox enzymes [19,20], free pool of metals [9], and nucleotides [4,5]. The whole set of biochemical reactions proceeds simultaneously, so that homeostasis is completely suppressed. The processes suppressing all functions of the organism increase in a geometric progression. All this leads to an increase in the toxicologic effect *in vivo*, *i.e.*, to lower effective concentrations of pollutants. This circumstance can explain a different order for a decrease in K_c for the pesticides under study and ATP and in the toxicity of these compounds. Nevertheless, a correlation between the toxicity and K_c values was found to be well pronounced for all representatives of aquatic life.

Table 4. Toxic effect of pesticides on different zooplankton species (LC_{50}, from literary data).

Pesticide	Common name	SCIENTIFIC NAME	Time h	Toxic dose, 10^{-6}						Ref
				g/l			M			
				Mean	Min	Max	Mean	Min	Max	
Roundup	Opossum shrimp	*Americamysis bahia*	96	40	31	53	0.24	0.18	0.31	[21]
	Water flea	*Scapholeberis kingi*	3	25	22	27	0.15	0.13	0.16	[22]
Setoxidim	Opossum shrimp	*Americamysis bahia*	96	141.8	-	-	0.43	-	-	[21
Basagran	Opossum shrimp	*Americamysis bahia*	96	132.5	-	-	0.55	-	-	[21]
	Water flea	*Scapholeberis kingi*	3	1425	-	-	5.93	-	-	[22]
Tilt	Opossum shrimp	*Americamysis bahia*	96	510	370	670	1.49	1.08	1.96	[21]

Table 5. Toxic effects of pesticides on crustacea (LC_{50}, from literary data).

Pesticide	Common name	Scientific name	Measurement	Time	Toxic dose (10^{-6}) g/l; M		Ref
Sencor	Northern pink shrimp	*PENAEUS DUORARUM*	Mortality	96 h	48.3	0.8229	[21]
	Fiddler crab	*UCA PUGILATOR*	Mortality	96 h	65	0.3033	[21]
2-Pyridine carbolic acid	Striped barnacle	*Balanus amphitrite*	Behavioral changes, general	24 h	20	0.1042	[23]
Roundup	Crayfish	*Orconectes nais*	Mortality	96 h	7	0.0514	[24]
	Crustacean subphylum	*Crustacea*	Abundance	16 d	98	0.5795	[25]
Basagran	Fiddler crab	*Uca minax*	Accumulation general	11 d	150 86	0.625 0.3579	[26]
Tachigaren	Crayfish/Crab order	*Decapoda*	Mortality	72 h	40	0.4036	[25]
Tilt	Crayfish	*Procambarus* sp.	Mortality	96 h	49	0.1432	[21]
	Crustacean subphylum	*Crustacea*	Abundance	16 d	92.5	0.27	[27]

Table 6. Toxicity of pesticides with respect to fishes (mortality—LC_{50}, from literary data).

Pesticide	Common name	Scientific name	Life stage	Time h	Toxic dose						Reference
					g/l (10^{-6})			M/l(10^{-6})			
					Mean	Min	Max	Mean	Min	Max	
Sencor	Bluegill	*Lepomis macrochirus*	Juvenile	96	75.96	60.6	97.4	0.354	0.283	0.454	[21]
		Lepomis macrochirus	0.8 g	96	92	68	124	0.429	0.317	0.579	[24]
	Channel catfish	*Ictalurus punctatus*	0.22 g	96	100	-	-	0.467	-	-	[24]
Rainbow trout,donaldson trout		*Oncorhynchus mykiss*	Juvenile	96	76.77	46	100	0.358	0.215	0.467	[21]
		Oncorhynchus mykiss	0.7 g	96	42	32.9	53.7	0.196	0.154	0.251	[21,24]
Sheepshead minnow		*Cyprinodon variegatus*	0.2 g	96	85	60	102	0.397	0.28	0.476	[21]
Kuzagard	*Cyprinus carpio*	*Cyprinus carpio*	8.9 cm, 25.2 g	48	191		-	0.558		-	[28]
Rainbow trout,donaldson trout		*Oncorhynchus mykiss*	6.8 cm, 4.5 g	48	174	-	-	0.509	-	-	[28]
Roundup	Bluegill	*Lepomis macrochirus*	0.9 g	96	135	113	162	0.798	0.668	0.958	[24,29, 30]
		Lepomis macrochirus	0.5 - 2.2 g	96	140	110	160	0.828	0.651	0.946	[30]
		Lepomis macrochirus	0.5 - 2.2 g	24	150	120	190	0.887	0.71	1.124	[30]
		Lepomis macrochirus	0.4 g	24	240	200	290	1.419	1.183	1.715	[24]
		Lepomis macrochirus	0.4 g	96	140	120	170	0.828	0.71	1.005	[24]
		Lepomis macrochirus	0.3 g	96	1.800	1.300	2.5	0.011	0.008	0.015	[24]
	Brown trout	*Salmo trutta*	No report	96	5.4	-	-	0.032	-	-	[31]
	Channel catfish	*Ictalurus punctatus*	0.5 - 2.2 g	96	130	110	160	0.769	0.651	0.946	[30]
		Ictalurus punctatus	0.2 g	96	4.4	3.7	5.2	0.026	0.022	0.031	[24]
	Cyprinus carpio	*Cyprinus carpio*	3.6 - 4.2 cm, 4.0 - 5.5 g	48	645	632	655	3.814	3.737	3.873	[32]
		Cyprinus carpio	No report	48	115	-	-	0.68	-	-	[33]
	Pink salmon	*Oncorhynchus gorbuscha*	juv. 2.6 MO, 4.3 cm, 0.5 g	48	94.000	-	-	0.556	-	-	[34]
Rainbow trout, donaldson trout		*Oncorhynchus mykiss*	juv. 2.6 MO, 4.3 cm, 0.5 g	96	10	-	-	0.059	-	-	[34]
		Oncorhynchus mykiss	juv. 2.6 MO, 4.3 cm, 0.5 g	96	93	-	-	0.55	-	-	[34]
		Oncorhynchus mykiss	0.5 - 2.2 g	96	140	120	170	0.828	0.71	1.005	[21,24, 29,30]
		Oncorhynchus mykiss	2.68 g	96	134	100	180	0.792	0.591	1.064	[21]
		Oncorhynchus mykiss	0.7 g	96	140	120	170	0.828	0.71	1.005	[24]
Sheepshead minnow		*Cyprinodon variegatus*	0.855 g	96	240	180	320	1.419	1.064	1.892	[21]
Western mosquitofish		*Gambusia affinis*	No report	48	6.2	5.5	7.1	0.037	0.033	0.042	[22]
Setoxidim	Bluegill	*Lepomis macrochirus*	no report	96	265	220	318	0.809	0.672	0.971	[21]
		Lepomis macrochirus	0.15 g	96	1.6	910	2.3	0.005	2.779	0.007	[21]
	Cyprinus carpio	*Cyprinus carpio*	5.5 cm, 1.8 g	48	360	-	-	1.099	-	-	[35]
Rainbow trout, donaldson trout		*Oncorhynchus mykiss*	No report	96	170	142	204	0.519	0.434	0.623	[21]
Sheepshead minnow		*Cyprinodon variegatus*	0.3 g	96	145.8	-	-	0.445	-	-	[21]
Basagran	Bluegill	*Lepomis macrochirus*	0.9 g	96	100	-	-	0.416	-	-	[21]
		Lepomis macrochirus	Juvenile	96	610	-	-	2.538	-	-	[21]
	Cyprinus carpio	*Cyprinus carpio*	No report	48	1420	-	-	5.909	-	-	[22]
Sheepshead minnow		*Cyprinodon variegatus*	0.58 g	96	136	-	-	0.566	-	-	[21]
Rainbow trout, donaldson trout		*Oncorhynchus mykiss*	Juvenile	96	190	-	-	0.791	-	-	[21]
		Oncorhynchus mykiss	2.6 g	96	100	-	-	0.416	-	-	[21]
Western mosquitofish		*Gambusia affinis*	No report	48	600	99	3600	2.497	0.412	14.98	[22,36]
Tachigaren	Bluegill	*Lepomis macrochirus*	0.39 g	96	100	-	-	1.009	-	-	[21]
	Pink salmon	*Oncorhynchus gorbuscha*	FRY, 1.3 g	48	120	-	-	1.211	-	-	[37]
Rainbow trout, donaldson trout		*Oncorhynchus mykiss*	0.95 g	96	100	-	-	1.009	-	-	[21]
Tilt	Bluegill	*Lepomis macrochirus*	No report	96	9.8	7.5	11.7	0.029	0.022	0.034	[21]
	Brouwn trout	*Salmo trutta*	No report	96	3.39	2.8	4.9	0.01	0.008	0.014	[21]
	Channel catfish	*Ictalurus punctatus*	No report	96	12	-	-	0.035	-	-	[21]
	Cyprinus carpio	*Cyprinus carpio*	No report	96	46	40	52	0.134	0.117	0.152	[21]
Rainbow trout, donaldson trout		*Oncorhynchus mykiss*	No report	96	830	670	1.3	2.425	1.958	0.004	[21]
		Oncorhynchus mykiss	Juvenile	96	13.2	11	16	0.039	0.032	0.047	[21]

Therefore, our data suggest that, upon ingress of Plt, pesticides, and their metal complexes in living organism, these compounds undergo chemical binding with ATP to give stable Plt-ATP complexes with their own K_c value. Such bioorganic complexes infringe energy metabolism in a living organism. The cells suffer from energy shortage, which leads to the death of the cells and sequentially of the entire organism. Such a mechanism of action manifests itself as a decrease in the enzymatic activity of bacteria or in the reproductive function of infusoria.

The established toxicity—K_c relationship was found to hold true for a wide variety of multicellular organisms. This relationship can be used for express-evaluation of pollutant toxicity towards representatives of aquatic life.

4. Conclusions

1) The toxicity factors of pesticides, viz., Lontrel, Sencor, Roundup, Bentazon, and Hymexazol and also Lontrel complexes with Cu, Co, Mn, Mg, Mo, Ni, and Zn towards the bacteria *Beneckea harveyi* and infusoria *Tetrahymena pyriformis* were measured.

2) The concentration dependence of the above toxicity factors was determined.

3) At any concentration, the toxicity of the pesticides was found to increase in the following order: Sencor > Lontrel > Roundup > Bentazon > Hymexazol, while that of the $MetL_2$ complexes is as follows: CuL_2 > CoL_2 > NiL_2 > MoL_2 > MnL_2 > ZnL_2 > MgL_2. The toxicity of $MetL_2$ complexes is higher (except for MgL_2) than that of free Lontrel.

4) Toxicity of that or another man-caused pollutant was found to be directly related to the energy shortage arising in cells under the action of pollutant complexes with ATP at the molecular level.

REFERENCES

[1] Yu. I. Skurlatov, G. G. Duka and A. Misiti, "Vvedenie v Ekologicheskuyu Khimiyu (Introduction to Ecological Chemiscry)," Vysshaya Shkola, Moscow, 1994, 400 p.

[2] E. A. Saratovskikh, N. B. Kozlova and V. V. Goncharov, "Pollution of Volga Water with Sewage of Kazan City," *Vodnye Resursy* (*Moscow*), Vol. 24, No. 1, 1997, pp. 56-60.

[3] E. A. Saratovskikh, N. B. Kozlova, V. G. Papin and E. V. Shtamm, "Biochemical and Photochemical Degradation of the Herbicide Lontrel," *Applied Biochemistry and Microbiology*, Vol. 42, No. 1, 2006, pp. 38-44. doi:10.1134/S0003683806010042

[4] E. A. Saratovskikh, T. A. Kondrat'eva, B. L. Psikha, R. I. Gvozdev and V. G. Kartsev, "Complex-Formation of Some Pesticides with Adenosine Triphosphoric Acid," *Bulletin of the Academy of Sciences of the USSR*, *Division of Chemical Science*, Vol. 37, No. 11, 1988, pp. 2252-2258. doi:10.1007/BF00959872

[5] E. A. Saratovskikh, M. V. Lichina, B. L. Psikha and R. I. Gvozdev, "Character of the Reaction of Dinucleotides and Polynucleotides with Some Pesticides," *Bulletin of the Academy of Sciences of the USSR*, *Division of Chemical Science*, Vol. 38, No. 9, 1989, pp. 1822-1827. doi:10.1007/BF00957770

[6] E. A. Saratovskikh, R. I. Papina and V. G. Kartsev, "Influence of Some Pesticides on Dicotyledonous and Cereals," *Sel'skokhoziaystvennaya Biologia*, Biologia Rastenii, Seria, Vol. 5, 1990, pp. 152-159.

[7] E. A. Saratovskikh, R. I. Papina and T. A. Kondrat'eva, "Deranged Metabolism of Cells during Germination of Dicotyledonous and Cereals under the Action of Pesticides and Metal Complexes," *Tsitologiya*, Vol. 41, No. 3-4, 1999, pp. 308-309.

[8] Z. G. Aliev, L. O. Atovmyan, E. A. Saratovskikh, V. I. Krinichnyi and V. G. Kartzev, "Synthesis, Structure, and Spectral Characteristics of Copper Complexes with Picolinic-Acid Derivatives," *Bulletin of the Academy of Sciences of the USSR*, *Division of Chemical Science*, Vol. 37, No. 11, 1988, pp. 2246-2252. doi:10.1007/BF00959871

[9] E. A. Saratovskikh, "Synthesis of Bidentate Complexes of 3,6-Dichloropicolinic Acid," *Bulletin of the Academy of Sciences of the USSR*, *Division of Chemical Science*, Vol. 38, No. 10, 1989, pp. 2140-2141. doi:10.1007/BF00962125

[10] "Method of Determining the Toxicity of Water and Water Extracts from the Soil, Sludge, Waste on Mortality and Fertility Change Daphnids," *Biological Control Methods*, Akvaros, Moscow, 2001.

[11] "Manual for Determining the Toxicity of Waters, Bottom Deposits, and Drilling Fluids by Biotesting," NIA-Priroda, Moscow, 2002.

[12] I. L. Tsvetkov and A. S. Konichev, "Ecological Biochemistry of Aquatic Life," Publishing of Moscow State University, Moscow, 2006, 104 p.

[13] D. M. Pampanin, I. Marangon, E. Volpato, G. Campesan and C. Nasci, "Stress Biomarkers and Alkali-Labile Phosphate Level in Mussels (Mytilus Galloprovincialis) Collected in the Urban Area of Venice (Venice Lagoon, Italy)," *Environmental Pollution*, Vol. 136, No. 1, 2005, pp. 103-107. doi:10.1016/j.envpol.2004.12.010

[14] E. A. Saratovskikh and A. I. Bokova, "Influence of Herbicides on the Population of Soil-Dwelling Collembolan," *Toxicological Review*, Vol. 5, 2007, pp. 17-23.

[15] T. W. Schultz, F. M. Applehans and G. W. Riggin, "Structure-Activity Relationships of Selected Pyridines. III. Log K_{ow} Analysis," *Ecotoxicology and Environmental Safety*, Vol. 13, No. 1, 1987, pp. 76-83. doi:10.1016/0147-6513(87)90044-3

[16] W. Pauli, S. Berger and S. Schmitz, "Validierung Toxikologischer Prufparameter an Tetrahymena: Membranfunktionen, Chemotaxis, Rotation im Elektrischen Drehfeld," FU-Berlin, Inst. für Biochemie und Molekularbiologie, UFO-Plan, F+E-Vorhaben, 106 03 083 (OECDG Data File), 1993.

[17] W. Pauli, S. Berger, L. Jaskulka and S. Schmitz, "A Case for the Inclusion of a Protozoan Test in Aquatic Toxicity

Assessment Using *Tetrahymena*," *Science of The Total Environment*, Vol. 134, No. S1, 1993, pp. 779-786.

[18] W. Pauli, S. Berger, S. Schmitz and L. Jaskulka, "Chemosensory Responses of *Ciliates*: A Sensitive End Point in Xenobiotic Hazard Assessment," *Environmental Toxicology and Water Quality*, Vol. 9, No. 4, 1994, pp. 341-346. doi:10.1002/tox.2530090414

[19] E. A. Saratovskikh, L. A. Korshunova, R. I. Gvozdev and A. V. Kulikov, "Inhibition of the Nicotinamide Adenine Dinucleotide-Oxidoreductase Reaction by Herbicides and Fungicides of Various Structures," *Russian Chemical Bulletin*, Vol. 54, No. 5, 2005, pp. 1322-1327. doi:10.1007/s11172-005-0401-6

[20] E. A. Saratovskikh, L. A. Korshunova, O. S. Roschupkina and Yu. I. Skurlatov, "Inhibition of the Nicotinamide Adenine Dinucleotide-Oxidoreductase Reaction by Metal Complexes," *Advances in Chemical Physics*, Vol. 26, No. 8, 2007, pp. 40-45.

[21] "Pesticide Ecotoxicity Database [Formerly: Environmental Effects Database (EEDB)]," Environmental Fate and Effects Division, US EPA, Washington DC, 2000.

[22] F. Sun, "Evaluating Acute Toxicity of Pesticides to Aquatic Organisms: Carp, Mosquito Fish and Daphnids," *Plant Protection Bulletin*, Vol. 29, No. 4, 1987, pp. 385-396.

[23] M. Kawamata, K. Kon-Ya and W. Miki, "Trigonelline, an Antifouling Substance Isolated from an *Octocoral Dendronephthya* sp.," *Fisheries Science*, Vol. 60, No. 4, 1994, pp. 485-486.

[24] F. L. J. Mayer and M. R. Ellersieck, 'Manual of Acute Toxicity: Interpretation and Database for 410 Chemicals and 66 Species of Freshwater Animals," Resource Publications, No. 160, US Department Interior, Fish and Wildlife Service, Washington DC, 1986.

[25] H. Okudaira, "Hymexazol. A New Plant Protecting Agent, V. Safety Tests," *Sankyo Kenkyusho Nempo*, Vol. 73, No. 25, 1973, pp. 48-51.

[26] G. M. Booth, C. C. Yu and D. J. Hansen, "Fate, Metabolism, and Toxicity of 3-Isopropyl-1*H*-2,1,3-Benzo-Thiadiazin-4(3*H*)-1-2,2-Dioxide in a Model Ecosystem," *Journal of Environmental Quality*, Vol. 2, 1973, pp. 408-411. doi:10.2134/jeq1973.00472425000200030025x

[27] D. O. Hessen, T. Kallqvist, M. I. Abdel-Hamid and D. Berge, "Effects of Pesticides on Different Zooplankton *Taxa* in Mesocosm Experiments," *Norwegian Journal of Agricultural Sciences*, No. 13, 1994, pp. 153-161.

[28] Z. Svobodova, J. Machova, R. Faina, A. Kocova and P. Kunce, "Acute Toxicity of Selected Pesticides to Aquatic Organisms," *Buletin Vyzkumny Uztav Rybarsky Hydrobiologicky Vodnany* (Prague), Vol. 22, No. 1, 1986, pp. 8-20.

[29] W. W. Johnson and M. T. Finley, "Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates," Resource Publications, Fish and Wildlife Service, USDI, Washington DC, 1980.

[30] L. C. Folmar, H. O. Sanders and A. M. Julin, "Toxicity of the Herbicide Glyphosate and Several of Its Formulations to Fish and Aquatic Invertebrates," *Archives of Environmental Contamination and Toxicology*, Vol. 8, No. 3, 1979, pp. 269-278. doi:10.1007/BF01056243

[31] K. J. Aanes, "Some Pesticides Used in Norwegian Agriculture and Their Environmental Effects on Common Inhabitants in Freshwater Ecosystems. Tolerance Limits," In: A. Helweg, Ed., *Pesticides in the Aquatic Environment*. *Appearance and Effects*, 12-14 November 1991, Tune Landboskole, Denmark, pp. 108-131.

[32] N. K. Neskovic, V. Poleksic, I. Elezovic, V. Karan and M. Budimir, "Biochemical and Histopathological Effects of Glyphosate on Carp, *Cyprinus carpio L*.," *Bulletin of Environmental Contamination and Toxicology*, Vol. 56, No. 2, 1996, pp. 295-302. doi:10.1007/s001289900044

[33] G. C. Li and C. Y. Chen, "Study on the Acute Toxicities of Commonly Used Pesticides to Two Kinds of Fish," *K'O Hsueh Fa Chan Yueh K'an*, Vol. 9, No. 2, 1981, pp. 146-152.

[34] W. T. Wan, R. G. Watts and D. J. Moul, "Effects of Different Dilution Water Types on the Acute Toxicity to Juvenile Pacific Salmonids and Rainbow Trout of Glyphosate and Its Formulated Products," *Bulletin of Environmental Contamination and Toxicology*, Vol. 43, No. 3, 1989, pp. 378-385. doi:10.1007/BF01701872

[35] Y. Nishiuchi, H. Iwamura and K. Asano, "Toxicity of Pesticides to Some Aquatic Animals. VI. Acute Toxicity of Latest Registered Pesticides to Some Aquatic Animals," *Seitai Kagaku*, Vol. 8, No. 1, 1985, pp. 13-15.

[36] T. S. Leung, S. M. Naqvi and C. LeBlanc, "Toxicities of Two Herbicides (Basagran, Diquat) and Algicide (Cutrine-Plus) to Mosquito Fish *Gambusia affinis*," *Environmental Pollution Series A*, *Ecological and Biological*, Vol. 30, No. 2, 1983, pp. 153-160. doi:10.1016/0143-1471(83)90012-0

[37] K. Kuroda, "Lethal Effect of Pesticides on Saghalien Trout Fry," *Water Purification Liquid Wastes Treatment*, Vol. 16, No. 5, 1975, pp. 441-448.

