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PROCESSES UNDERLINING THE ACTION OF PESTICIDES ON ECOSYSTEMS AND HUMAN ORGANISM

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Abstract. A brief overview of the works carried out over a long period is presented. The role of adenosine triphosphate (ATP) on the mechanism of action of pesticides and pesticide complexes with metals is shown. The results of investigation of the effect of pesticides and their complexes with metals on the enzyme systems and nucleotides are presented. The results are summarized in a single system of interrelated and interdependent processes developing *in vivo* in the form of a branched biological mechanism.

Keywords: pesticides, adenosine triphosphate, NADH-oxidoreductase, nucleotides.

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Introduction

In 1986 the United Nations Organization conference on the environment and development had to concede that pesticides are predominant contaminants of the environment [1]. The use of "plant protection chemicals," in particular, pesticides and herbicides, contributes to the yield [2,3] and has already led to significant negative consequences.

Pesticides as a whole and herbicides in particular are substances with high biological activity. They can exert a toxic effect on many components of cells: enzymes, structural and functional proteins, lipoproteids, polysaccharides, nucleic acids, and others. The elucidation of the mechanism of the toxic effect is an important challenge, the solution of which would allow one to establish the real and potential danger of application of these or other compounds for human and non-target organisms. Despite the enormous scale of production and use of chemical facilities for cultivated plant protection, many data on the mechanism of their action still remain unknown. It is considered that, probably, each pesticide acts via a unique mechanism. For example, the acting components of pesticides, namely, zenkor, lontrel, roundup, kusagard, setoxidim, basagran, tilt, and tachigaren, belong to different classes of chemical compounds. According to available literature data (Table 1), they interact with various enzymatic systems, have their own specific binding sites, and are characterized by different mechanisms of action.

Much data concerning the influence of herbicides and fungicides on various components of the living cell [15-17], in particular, on some enzymes [18-21] have been reported. For instance, anticholinesterase compounds**,** organophosphorus pesticides, carbamates and triazines [22] are structurally similar to substrates and competitively inhibit their activity. The effect was evaluated for fries of Mediterranean fishes *Dicentrarchus labrax* [23] and for rats (*Maple amber*) fed with soybean after treatment with zenkor and atrazine [18]. It was shown that herbicide basagran suppressed the antiphosphatecholinesterase activity and resulted in an increase in the hydroxylase activity [9].

The growth of fungi *Cryptococcus neoformans* was suppressed by glyphosate due to the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase [24]. A non-productive four-membered complex is formed between the enzyme, pesticide, and phosphate [25]. The octahedral coordination mode is formed by the metal ion: Co glyphosate enzyme as that in 3-deoxy-D-arabiheptulosonate-7-phosphate synthase localized in cytosols [26].

Oxidative phosphorylation is performed by Zn-containing enzymes. Dinoseb, dichlorodiphenyltrichloroethane, sevin and pentachlorophenol separate oxidative phosphorylation in mitochondria of *Palma christi* [27] and decrease the ATP content in glycols of soybean [28]. Chlorine-containing organic pesticide endosulfan reacts with glutathione

(cofactor of glutathione peroxidase), considerably decreasing the activity of the enzyme. The loss of secretory reactions in thylakoids of adrenocortical steroidogenic cells and changes in the enzyme activity indicate that the pesticide was involved in the oxidative reactions [29].

The formation of complexes of vegetable peroxidase with various substrate-inhibitors was established [30]. Both the direct participation of the metal in the substrate addition to the protein part of the molecule and providing a relationship between the flavine group and apoenzyme under the action of the metal are assumed. The neighborhood of the pyridine nitrogen atom to the carboxyl group in picolinic acid (picloram) is manifested as the ability to complexation and metal removal from enzymes [31].

The tests on human and rat tissues showed that tachigaren and its metabolites (four enzymes synthesizing pyrimidine) inhibit mitochondrial [32]. This results in the changes in the pyridine–nucleotide pool that provides the work of immune cells. The reaction is reversible and its mechanism is uncompetitive with respect to the substrate and cofactor ubiquinone [10]. On the other hand, diverse xenobiotics, both pesticides and metals, are abundant in considerable amounts in the nature, namely, in air, soil, and water [10,19,20]. If these xenobiotics get into the human organism, they may cause various diseases [21,24]. In the presence of pesticides with the ligand properties, their combined effect on living organisms can be enhanced or weakened.

The ability of the environment to self-purification, *i.e.*, decomposition of contaminants, is determined, to a great extent, by the occurrence of enzymatic redox processes in cells of plants and microorganisms.

For a long time, we have studied the mechanism of the effect of the following pesticides: zencor, lontrel, roundup, kusagard, setoxidim, bazagrane, tilt, and tachigaren. The acting components of these preparations belong to various classes of chemical compounds; according to available literature data, they are characterized by different mechanisms of action (Table 1). However, we were able to show that the action of these compounds is multifunctional and is not restricted by the properties listed in Table 1. They exhibit a significantly broader range of activity.

Complex formation between pesticides and metals

Metal complexes of lontrel (L) were not studied by our research group only. Previously, we showed that 3,6-dichloropicolinic acid (DCPA) with the active principle of the herbicide lontrel readily formed complexes with metals, which are the major environmental pollutants and stable under natural conditions [33,34].

We have shown, for the first time [33], that the bidentate complexes, or chelates with microelements, are formed in cells of living organisms (Figure 1). In all cases, 1 : 2 complexes are formed. The complex is formed *via* a strong covalent metal-oxygen bond of the type O-M-O and metal-nitrogen bond (M-N). According to IR spectra, the strength of the complexes changes in the order: $NiL_2 > FeL_2 > MoL_2 = CoL_2 > CuL_2 > MnL_2 > ZnL_2 > MgL_2$.

Table 1

Mechanism of action of pesticides.

Trivial name	*Field of application*	*Mechanism of action*	*Ref.*
Zenkor, Metribuzin	selective to dicotyledons and solanaceous	complexes with membrane lipids	[4]
Lontrel, Clopyralid	a wide spectrum of action	similar to auxin	[5]
Kusagard	selective to dicotyledons, beet, cotton	lesion meristem tissues	[6]
Roundup, Glyphosate	for control of perennial weeds	inhibitor of enolpyruvateshe-kemate-phosphate synthase	[7]
Setoxidim	selective to dicotyledons, beet, solanaceous		
Basagran, Bentazon	selective to grains	inhibitor of photo- ; protein- ; lipids- ; RNA - synthesis	[8,9]
Tachigaren, Hymexazol	selective to grass, sugar beet	inhibitor of dehydrogenase (mitochondrial)	[10]
Tilt, Propiconazole	fungicide, selective to grain crops, to rape	7-etoxyrezofurine O-diethylase; inductor glutathione S-transferase	[11,12]
Lontrel metal complexes	a wide spectrum of action	inhibitor of NADH-oxidoreductase	[13,14]

These X-ray diffraction pattern of the lontrel complex with copper shows that the complexes of this type have an octahedral structure with various degrees of distortion of the coordination polyhedron as shown in Figure 1.

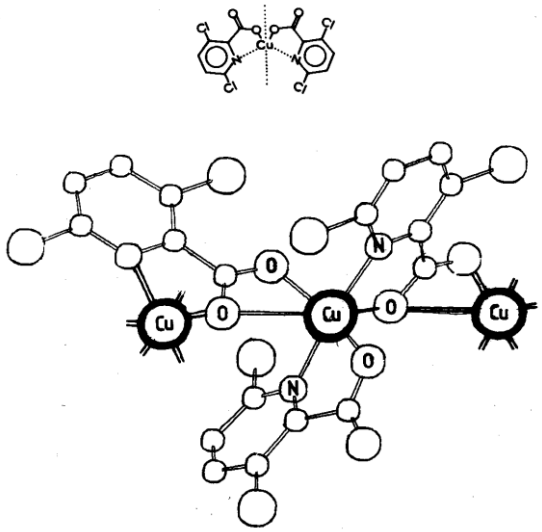

Figure 1. Structure of the CuL_2 complex, according to X-ray diffraction data [33]. $NiL_2 > FeL_2 > MoL_2 = CoL_2 > CuL_2 > MnL_2 > ZnL_2 > MgL_2$

According to the ESR data [35], under the native conditions, the considered complexes exist as a single whole in the non-dissociated state. They can participate in further complex formation with bioactive ligands due to the filling of the coordination sphere of the metal. They are capable of participating in further complex formation with bioactive ligands due to the filling of the coordination sphere of the metal.

Reactions of the pesticides with adenosine triphosphoric acid and NADH

We have proved for the first time [36] that pesticides themselves and their metal complexes react with mono- and dinucleotides. The structures of the adenosine triphosphoric acid (ATF) and *ε*-ATF complexes with lontrel and its metal complex are shown in Figure 2. In all cases, two- or three-component complex systems are formed. It was shown that the pesticide complex with ATF is formed due to the protonation of the N-7 nitrogen atom of the adenine heterocycle, and the nitrogen atom of the terminal NH_2 group can simultaneously be bound to the pesticide molecule due to the formation of a hydrogen bond.

The interaction of pesticides (P) with ATP (A) occurs according to the scheme:

$$A + nP \leftarrow k^+/k^- \rightarrow \text{complex}.$$

$K_{c/form} = k^+/k^-$ is the complex formation constant.

The value of the stability constants of these complexes were determined by the equation:

$$K_{c/form} = ([A_o] - [A])/([A]\{[P_o]\text{-}n([A_o]\text{-}[A])\}^n).$$

The stoichiometric coefficient *n* for all the compounds studied was determined as equal to 1 ± 0.2. One molecule of ATP reacts with one molecule of pesticide. The obtained values for the complex formation constants ($K_{c/form}$) are presented in Table 2.

The studied compounds are characterized by two series of activity according to the complex formation constants ($K_{c/form}$):

(1) sencor > lontrel > kusagard > roundup > setoxidim > basagrane > tachigaren > tilt;

(2) $CuL_2 > CoL_2 > NiL_2 > L \approx FeL_2 \approx ZnL_2 \approx MoL_2 >> MgL_2 \approx MnL_2$.

The formation of the pesticide complexes with ATP results in an energy deficiency in the tissues of organisms [37-40]. The effect of pesticides and their metal complexes induces the energy deficiency of the cell, namely, inhibition of energy metabolism due to the formation of a complex with adenosine triphosphoric acid.

It is known that polynucleotides, particularly, pyridinenucleotides, form complexes of various types, including charge-transfer complexes, and are highly reactive towards a series of metals [41]. However, the introduction of the etheno group does not almost change the electronic structure of the nucleotide fragment of a NADH molecule.

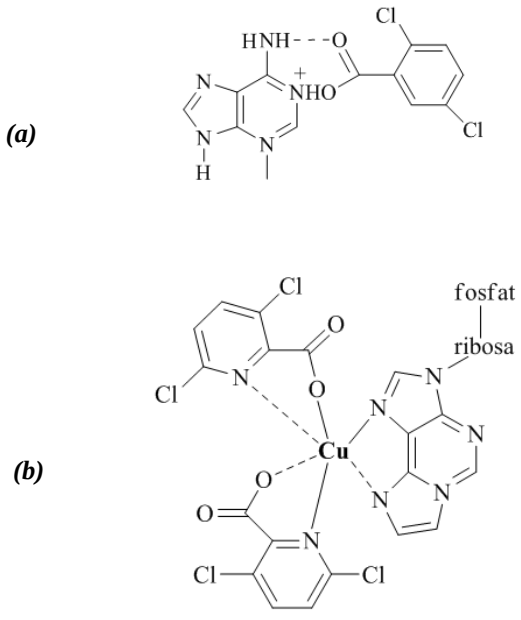

Figure 2. Structure of *(a)*-the ATF-lontrel complex [36] and *(b)* -the *ε*-ATF with metal complex of lontrel [*ε*-ATF-CuL_2] [36]. Here, CuL_2 is the DCPA complex synthesized by us [33,34].

Table 2

Complex formation constants of the nucleotides with pesticides and herbicide lontrel metal complexes ($K_{c/form}$, M^{-1}) [36,42].

Pesticides	$K_{c/form}$ 10^{-3}, M^{-1} *ε-ATP*	$K_{c/form}\cdot10^{-3}$, M^{-1} *ε-NADH*	*Lontrel metal complexes*	$K_{c/form}\cdot10^{-3}$, M^{-1} *ε-ATP*	$K_{c/form}\cdot10^{-3}$, M^{-1} *ATP*	$K_{c/form}\cdot10^{-3}$, M^{-1} *ε-NADH*
Basagrane	4.7±0.4	-	MgL_2	0.8±0.02	21.1±14.6	-
Sencor	26.5±3.3	21.33±1.5	MnL_2	2.2±0.1	40.4±14.8	-
Kusagard	9.7±0.5	2.51±0.04	FeL_2	8.8±0.4	47.2±20.3	0.55±0.06
Lontrel (L)	15.0±2.0	11.70±0.4	CoL_2	600±200	32.2±0.6	3.05±0.14
Roundup	8.2±1.2	2.20±0.41	NiL_2	21.6±0.5	105.8±54	4.74±0.34
Setoxidim	5.0±0.3	2.84±0.71	CuL_2	851.4±82	296.6±90	4.56±0.16
Tachigaren	1.1±0.04	1.80±0.39	ZnL_2	1.6±0.06	86.3±18	-
Tilt	0.8±0.06	0.46±0.06	MoL_2	3.6±0.4	-	2.15±0.07

Therefore, the complexation of pesticides with NADH was concluded on the basis of the value of fluorescence quenching of its chemical analog, modified dinucleotide *ε*-NADH in which the adenine fragment is subjected to etheno modification [42,43].

Figure 3 shows the dependences of the fluorescence intensity of *ε*-NADH on the concentration of various quenchers. When the concentration of pesticide (or metal complex) increases, the fluorescence quenching of compound *ε*-NADH is observed, which is not accompanied by a shift of the position of the excitation maximum and fluorescence emission. The absence of spectral changes in all considered cases indicates the absence of changes in the ground and excited levels of the modified compounds formed by the reactions with pesticides.

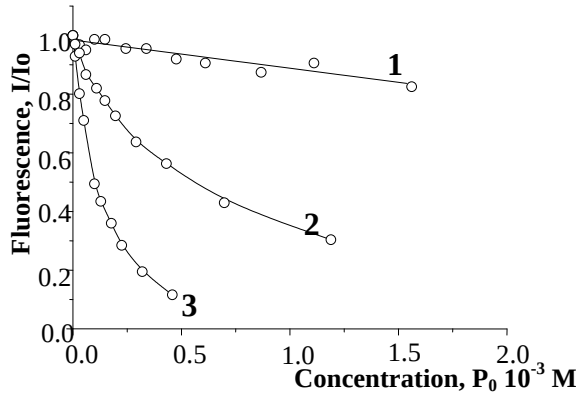

Figure 3. Dependences of the fluorescence intensity of *ε*-NADH on the pesticide concentration: *(1)* tilt*; (2)* kusagard*; (3)* zenkor [43]. The concentration of *ε*-NADH is $1\cdot10^{-5}$ M. Points are experimental data and solid lines are theoretical curves.

Fluorescence quenching was observed at the concentrations of the pesticide and lontrel metal complexes ranging from 10^{-6} to 10^{-3} M. Such low concentrations of the quencher exclude the assumption that the quenching proceeds *via* the Stern–Volmer mechanism due to random collisions. Therefore, the result of quenching is the formation of a covalent bond with the adenine fragment, as it is shown in the scheme of the [NADH–L] complex presented in Figure 4.

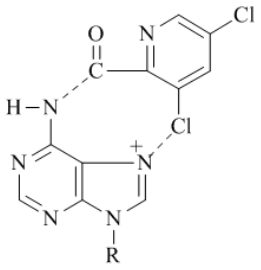

Figure 4. Structure of the NADH-lontrel complex [*ε*-NADH-L] [43].

The mathematical model of the process was considered to refine the mechanism of formation of complexes [*ε*-NADH–pesticide] and to estimate their stability constants, as the complex formation constants ($K_{c/form}$), as well as for the ATP (Table 2).

As can be seen from Table 2, among the synthesized pesticides, zenkor has the lowest complexation constant ($K_{c/form}$) for the complex with *ε*-NADH ($K_{c/form}$ = $2.1\cdot10^4$ M^{-1}) and tilt has the highest one ($K_{c/form}$ = $4.6\cdot10^2$ M^{-1}). It is noteworthy that the complexation constant of the lontrel metal complexes with *ε*-NADH is substantially lower than the corresponding constant for lontrel. It is known than in solution NADH exists predominantly in a folded conformation in which the adenine moiety of the molecule is localized near the nicotine amide moiety of the nucleotide [44]. About 90% of dinucleotide exist in this conformation in solution. The rest 10% exist in solution in the "open" conformation when the nicotine amide moiety is removed from the adenine structure. Therefore, it can be assumed that a decrease in the complexation constants with *ε*-NADH for the

metal complexes compared to lontrel indicates steric hindrances appeared upon the formation of the [NADH–ML_2] complex. In addition, we were able to determine a relationship between genotoxicity of the investigated pesticides and their complex formation constants with dinucleotide NADH [14].

Effect of pesticides on the activity of NADH-oxidoreductase as oxidative enzyme

The results of our research regarding the action of the pesticides of the enzyme systems are presented in the works [13,39,43,45]. The effect of pesticides on the activity of NADH-oxidoreductase (NADH-OR) is illustrated in Table 3. The experimental kinetic curves for the rate of NADH-OR oxidation *vs.* the concentration of the substrate were converted to the Lineweaver–Burk coordinates. All studied pesticides inhibit NADH-OR but show different types of inhibition.

Of all the compounds studied, the highest inhibitory activities were found for zenkor and bazagran. The inhibitory ability of CuL_2, MoL_2, and FeL_2 is higher than that of the original lontrel. The Michaelis constants (K_m) calculated without an inhibitor are $6.6\cdot10^{-4}$ and $2.47\cdot10^{-3}$ mol L^{-1} for NADH and NT, respectively. Lontrel, zenkor, kuzagard, tachigaren and CuL_2, FeL_2, MnL_2, MoL_2 inhibit the reduction of an electron donor in the competitive manner. Apparently, being its structural analog, a pesticide binds to the enzyme in the binding site with the formation of the NADH nonproductive complex. Setoxidim, roundup, tilt, MgL_2, NiL_2, ZnL_2, and CoL_2 inhibit the reduction of NADH-OR from NADH in the uncompetitive manner, apparently, due to the nonspecific interaction with the protein matrix outside the enzyme active site.

In terms of the K_i values with respect to NADH, the herbicides and lontrel complex with metals can be arranged in the following activity order: CuL_2<MoL_2<zenkor<lontrel<FeL_2<MnL_2 <ZnL_2<NiL_2<MgL_2<basagran<CoL_2<kusagard<tachigaren<roundup<tilt<setoxidim. This series is similar to that of complexation constants of these compounds with NADH given in Table 2.

Lontrel, zenkor, basagran, and roundup inhibit the reduction of NT in the uncompetitive manner, apparently, due to the nonspecific interaction with the protein matrix outside the enzyme active center. This interaction could induce conformational changes around the electron transfer site, resulting in the inhibition of enzymatic activity. Meanwhile, kusagard, setoxidim, tilt, and tachigaren compete with NT for the binding region on the enzyme. These differences can be due to different structures of the examined pesticides.

Table 3

Effect of inhibitors on NADH-oxidoreductase [13,43,45].
(NADH-OR from methane oxidation bacteria *Methylococcus capsulatus* (strain M).

Pesticide	I_{50} *mol* L^{-1}	*for NADH*				*for NT*			
		V_{max} *mol* L^{-1} s^{-1}	S_1 *mol* L^{-1}	$K_i\cdot10^4$ *mol* L^{-1}	*Type*	V_{max} *mol* L^{-1} s^{-1}	S_2 *mol* L^{-1}	$K_i\cdot10^4$ *mol* L^{-1}	*Type*
Zencor	$5.00\cdot10^{-4}$	-	$4.93\cdot10^{-3}$	0.25	A	$0.23\cdot10^{-6}$	$3.39\cdot10^{-4}$	8.94	B
Lontrel (L)	$1.10\cdot10^{-3}$	-	$1.23\cdot10^{-3}$	1.00	A	$1.88\cdot10^{-6}$	$6.98\cdot10^{-4}$	7.42	B
Bazagran	$6.00\cdot10^{-4}$	$1.82\cdot10^{-6}$	$1.83\cdot10^{-4}$	12.80	B	$0.26\cdot10^{-6}$	$2.55\cdot10^{-4}$	8.40	B
Kuzagard	$27.0\cdot10^{-2}$	-	$9.86\cdot10^{-3}$	14.00	A	-	$5.72\cdot10^{-3}$	158.9	A
Tachigaren	$2.70\cdot10^{-3}$	-	$2.47\cdot10^{-3}$	21.00	A	-	$5.30\cdot10^{-3}$	4.55	A
Roundup	$1.70\cdot10^{-3}$	$3.33\cdot10^{-6}$	$6.17\cdot10^{-4}$	22.00	C	$0.21\cdot10^{-6}$	$2.00\cdot10^{-4}$	42.90	B
Tilt	$2.20\cdot10^{-3}$	$1.25\cdot10^{-4}$	$5.98\cdot10^{-4}$	23.00	C	-	$13.00\cdot10^{-3}$	1.52	A
Setoxidim	$17.0\cdot10^{-2}$	$2.00\cdot10^{-6}$	$7.59\cdot10^{-4}$	397.5	C	-	$11.00\cdot10^{-3}$	8.04	A
MgL_2	$2.00\cdot10^{-3}$	$1.66\cdot10^{-6}$	$8.97\cdot10^{-4}$	12.67	B	-	$23.83\cdot10^{-3}$	3.55	A
MnL_2	$3.00\cdot10^{-3}$	-	$4.93\cdot10^{-3}$	3.80	A	$1.72\cdot10^{-6}$	$1.81\cdot10^{-3}$	22.30	D
ZnL_2	$1.00\ 10^{-3}$	$2.00\cdot10^{-6}$	$8.22\cdot10^{-4}$	10.19	B	$1.10\cdot10^{-6}$	$1.72\cdot10^{-3}$	2.46	D
CuL_2	$3.30\cdot10^{-4}$	-	$32.88\cdot10^{-3}$	0.06	A	$0.44\cdot10^{-6}$	$9.37\cdot10^{-4}$	4.01	B
CoL_2	$1.50\cdot10^{-3}$	$2.20\cdot10^{-6}$	$7.89\cdot10^{-4}$	13.73	B	$1.68\cdot10^{-6}$	$1.40\cdot10^{-3}$	13.10	D
NiL_2	$2.00\cdot10^{-3}$	$1.80\cdot10^{-6}$	$1.23\cdot10^{-3}$	12.36	B	$1.10\cdot10^{-6}$	$3.11\cdot10^{-3}$	11.70	B
FeL_2	$1.10\cdot10^{-3}$	-	$8.97\cdot10^{-3}$	1.13	A	$1.54\cdot10^{-6}$	$2.20\cdot10^{-3}$	11.70	B
MoL_2	$8.50\cdot10^{-4}$	-	$19.72\cdot10^{-3}$	0.13	A	-	$47.62\cdot10^{-3}$	0.41	A

In the absence of an inhibitor,
$V_{max} = 7.40\cdot10^{-6}$ *mol* L^{-1} s^{-1},
$S_1 = 6.58\cdot10^{-3}$ *mol* L^{-1},
$S_2 = 2.65\cdot10^{-3}$ *mol* L^{-1};
Type of inhibition: A is competitive, B is uncompetitive, C is noncompetitive, D is mixed.

The metal complexes of lontrel are known to exhibit herbicide activities *in vivo* [39,43]. In addition, as noted above, the complex formed by the herbicide lontrel with the copper ion exhibits a much higher inhibitory activity than the starting lontrel. Therefore, we carried out an additional study of a series of complexes of these pesticides with different doubly charged metal ions, ML_2, and salts of these metals. Among the lontrel complexes with metal ions, only the complexes with Mg and Mo were proved to be competitive reductase inhibitors with respect to NT. The lontrel complexes with Cu, Ni, and Fe ions inhibit the enzyme noncompetitively, while the lontrel complexes with Mn, Zn, and Co display a mixed type of inhibition. The difference between the inhibition patterns may be related to the difference between the acceptor abilities of the metal ions.

The metal complexes are characterized by the predominant influence of the ligand environment. The pyridine ring has a structure close to NADH, *i.e.*, is able to replace the substrate on the protein, and the nitrogen atom may donate an unshared pair of electrons. A change in the coordination sphere of the metal (ligand environment) leads to fundamental changes in the nature of inhibition. It is known [46,47] that the 2Fe-2S cluster is in the composition of the active center of NADH-OR. Being in the composition of the complex, the metal cannot act as a free cation, since it is significantly affected by the ligand environment. The considered ligand (lontrel) is capable of occupying the site of NADH, donor of two electrons, in the active center of the enzyme. Probably, the interaction with iron of the cluster occurs through the carboxyl of the ligand and due to a high electron density of chloropyridine. As a result, the ligand or complex inhibits the NADH-binding region of the electron-transfer chain. The structures of the complexes allow them to play the role of both the electron donor and electron acceptor. It can be assumed that the complexes form chains in which the ligands act as a "bridge": L- M-L-Fe-OR and the metal of the complex pulls electrons from the 2Fe-2S cluster of the active center of the enzyme as it shown in Figure 5.

Figure 6 schematically shows the direction of the inhibitor attack. The intramolecular electron transfer in the active center of NADH-OR proceeds from flavine adenine dinucleotide (FAD) to the iron-sulfur cluster 2Fe-2S and further to an artificial electron acceptor [25,26,48]. For competitive inhibition, the pesticide or ML_2 occupies the site of NT and thus breaks the electron transfer chain.

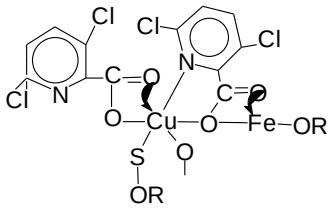

Figure 5. Complex ML_2 in the active center of the NADH-OR [45].

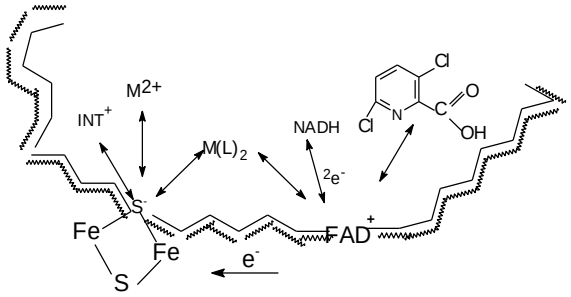

Figure 6. D**irections of the attack of various inhibitors [46].**

Conclusions

These studies have shown that pesticides are substances with a high ability to form complex compounds of superior chemical stability. These complex compounds show no selective action. Pesticides in a related form are transferred through trophic pathways and enter the body. Pesticides inhibit the biological activity of oxidizing enzymes. As a result, the organism and the whole environment lose the ability of degradation of toxic substances. There is a gradual accumulation of pesticides in the bodies of living beings and the environment. The accumulation of pesticides primarily leads to metabolic failure of energy metabolism due to the occurrence of energy deficiency because of binding of ATP with pesticides.

The sum of these effects is the cause of almost all diseases of modern man, including cancer. It is very necessary to forbid the use of pesticides for safety of our world, ecosystems, and human being and for population health improvement.

References

1. Koptyug, V.A. UNO Conference on Environment and Development (Rio de Janeiro, June 1992): Informational Review. SB RAS: Novosibirsk, 1992, 62 p. (in Russian).

2. Skurlatov, Yu.I.; Duka, G.G.; Misiti, A. Introduction to Ecological Chemistry. Vysshaya Shkola: Moscow, 1994, 400 p. (in Russian).
3. Yablokov, A.V. Poisonous Dressing. Problems of Application of Toxic Chemicals and Ways to Ecological Agriculture. Mysl': Moscow, 1990, 125 p. (in Russian).
4. Ziegler, W. Ionenkanäle in planaren bimolekularen lipidmembranen, erzeugt durch das herbizid sencor 7OWP. Biológia (ČSSR), 1982, 37(11), pp. 1071-1077.
5. Hall, J.C.; Bassi, P.K.; Spencer, M.S.; Vanden Born, W.H. An evaluation of the role of ethylene in herbicidal injury induced by picloram and clopyralid in rapeseed and sunflower plants. Plant Physiology, 1985, 79, pp. 18–23. DOI: 10.1104/pp.79.1.18.
6. Iwataki, I.; Hirono, Y. The chemical structure and herbicidal activity of alloxidim-Na and related compounds. Fifth International Union for Pure and Applied Chemistry (IUPAC) Congress Zürich; Verlag: Zürich, 1978, Vol. Abstr., No. 11 – 12.
7. Amrhein, N.; Deus, B.; Gehrke, P.; Steinrücken, H.C. The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. Plant Physiology, 1980, 66(5), pp. 830-834. DOI: 10.1104/pp.66.5.830.
8. Trebst, A.; Wietoska, H. Hemmung des photosynthetischen elektronentransports von chloroplasten durch metribuzin. Zeitschrift für Naturforschung, 1975, 30(7/8), pp. 499-504. DOI: 10.1515/znc-1975-7-813.
9. Al-Mendofi, O.; Ashton, F.M. Bentazon influence on selected metabolic processes of isolated bean leaf cells. Journal of Plant Growth Regulation, 1984, 3(2), pp. 121–126.
10. Knecht, W.; Löffler, M. Species-related inhibition of human and rat dihydroorotate dehydrogenase by immunosuppressive isoxazol and cinchoninic acide derivatives. Biochemical Pharmacology, 1998, 56(9), pp. 1259-1264. DOI: https://doi.org/10.1016/S0006-2952(98)00145-2.
11. Levine, S.L.; Oris, J.T. Enhancement of acute parathion toxicity to fathead minnows following pre-exposure to propiconazole. Pesticide Biochemistry and Physiology, 1999, 65(2), pp. 102-109. DOI: https://doi.org/10.1006/pest.1999.2434.
12. Egaas, E.; Sandvik, M.; Fjeld, E.; Kallqvist, T.; Goksoyr, A.; Svensen, A. Some effects of the fungicide propiconazole on cytochrome P450 and glutathione S-transferase in brown trout (Salmo trutta). Comparative Biochemistry and Physiology. Part C: Pharmacology, Toxicology and Endocrinology, 1999, 122(2,3), pp. 337-344. DOI: https://doi.org/10.1016/S0742-8413(98)10133-0.
13. Saratovskikh, E.A.; Korshunova, L.A.; Gvozdev, R.I.; Kulikov, A.V. Inhibition of the nicotinamide adenine dinucleotide-oxidoreductase reaction by herbicides and fungicides of various structures. Russian Chemical Bulletin, 2005, 54(5), pp. 1322-1326. DOI: 10.1007/s11172-005-0401-6.
14. Saratovskikh, E.A.; Glaser, V.M.; Kostromina, N.Yu. Kotelevtsev, S.V. Genotoxicity of the pesticide in ames test and the possibility to formate the complexeses with DNA. Ecological genetics, 2007, 5(3), pp. 46-55, (in Russian).
15. Fedtke, C. Biochemistry and Physiology of Herbicide Action. Springer-Verlag: Berlin-Heidelberg-NewYork, 1982, 223 p.
16. Kadyrov, Ch.Sh. Gerbicides and Fungicides as Antimetabolites and Inhibitors of Enzyme Systems. Fan: Tashkent, 1970, 83 p. (in Russian).
17. Fudel-Osipova, S.I. Ed. Physiological and Biological Mechanism of Pesticide Action. Naukova dumka: Kiev, 1981, 256 p. (in Russian).
18. Mathew, R.; Kacew, S.; Khan, S.U. Bioavailability in rats of bound pesticide residues from tolerant or susceptible varieties of soybean and canola treated with metribuzin or atrazine. Chemosphere, 1998, 36(3), pp. 589-596. DOI: https://doi.org/10.1016/S0045-6535(97)00367-6.
19. Banas, A.; Banas, W.; Stenlid, G.; Stymne, S. Selective increase in acyl hydrolase activity by graminicides in whea. Biochemical Society Transactions, 2000, 28(6), pp. 777-779. DOI: 10.1042/bst0280777.
20. Gruys, K.J.; Marzabadi, M.R.; Pansegrau, P.D.; Sikorski, J.A. Steady-state kinetic evaluation of the reverse reaction for *Escherichia coli* 5-enolpyruvoylshikimate-3-phosphate synthase. Archives of Biochemistry and Biophysics, 1993, 304(2), pp. 345-351. DOI: https://doi.org/10.1006/abbi.1993.1360.
21. Nosanchuk, J.D.; Ovalle, R.; Casadevall, A. Glyphosate inhibits melanization of *Cryptococcus neoformans* and prolongs survival of mice after systemic infection. The Journal of Infectious Diseases, 2001, 183(7), pp. 1093-1099. DOI: https://doi.org/10.1086/319272.
22. Grin, D.; Goldberger, R. Molecular aspects of life. Mir: Moscow, 1968, 400 p. (in Russian).
23. Varo, I.; Navarro, J.C.; Amat, F.; Guilhermino, L. Effect of dichlorvos on cholinesterase activity of the European sea bass (*Dicentrarchus labrax*). Pesticide Biochemistry and Physiology, 2003, 75(3), pp. 61-72. DOI: https://doi.org/10.1016/S0048-3575(03)00019-1.
24. Kiyomiya, K.; Matsushita, N.; Matsuo, S.; Kurebe, M. Cephaloridine-induced inhibition of cytochrome C oxidase activity in the mitochondria of cultured renal epithelial cells (LLC-PK(1)) as a possible mechanism of its nephrotoxicity. Toxicology and Applied Pharmacology, 2000, 167(2), pp. 151-156. DOI: https://doi.org/10.1006/taap.2000.8981.
25. Du, W.; Wallis, N.G.; Payne, D.J. The kinetic mechanism of 5-enolpyruvyl-shikimate-3-phosphate synthase from a gram-positive pathogen *Streptococcus pneumonia*. Journal of Enzyme Inhibition and Medicinal Chemistry,

2000, 15(6), pp. 571-581. DOI: http://dx.doi.org/10.3109/14756360009040711.
26. Ganson, R.J.; Jensen, R.A. The essential role of cobalt in the inhibition of the cytosolic isozyme of 3-deoxy-*arabino*-heptulosonate-7-phosphate synthase from *Nicotiana silvestris* by glyphosate. Archives of Biochemistry and Biophysics, 1988, 260(1), pp. 85-93. DOI: https://doi.org/10.1016/0003-9861(88)90427-4.
27. Kuz`minskaya, U.A. Biochemical characterization of subcellular structures of the liver under the influence of pesticides. (The mechanism of action of organochlorine and urea pesticide.). Ph.D. Thesis, Kiev, Ukraine, 1975. (in Russian)
28. Gruenhagen, R.D.; Moreland, D.E. Effect of herbicides on ATP levels in excised soybean hypocotyls. Weeds, 1971, 19(4), pp. 319-323.
29. Dorval, J.; Leblond, V.S.; Hontela, A. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to endosulfan, an organochlorine pesticide. Aquatic Toxicology, 2003, 63(3), pp. 229-241. DOI: https://doi.org/10.1016/S0166-445X(02)00182-0.
30. Ugarova, N.N.; Lebedeva, O.V. The structure and functions of horseradish peroxidase. Biochemistry, 1978, 43(10), pp. 1731-1742, (in Russian).
31. Shcheglov, Yu.V.; Sokolov, M.C.; Kasihin, A.N. Herbicidal activity and the synthesis of piclorame and some other derivatives of 2-picoline. Agrochemistry, 1967, 5, pp. 105-111.
32. Schomburg, D.; Salzmann, M.; Stephan D. Eds. Enzyme Handbook: Volume 6: Class 1.2–1.4: Oxidoreductases. EC 1.3.99.11. Springer-Verlag: Berlin Heidelberg, 1993, XVI, 918 p.
33. Aliev, Z.G.; Atovmyan, L.O.; Saratovskikh, E.A.; Krinichnii, V.I.; Kartsev, V.G. 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 (Russian Chemical Bulletin). (Izvestiya of the Academy of Sciences of the USSR. Division of Chemical Science), 1988, 37(11), pp. 2246-2252, (in Russian). DOI: 10.1007/BF00959871.
34. Saratovskikh, E.A. Synthesis of bidentate complexes of 3,6-dichloropicolinic acid. Bulletin of the Academy of Sciences of the USSR, Division of Chemical Science (Russian Chemical Bulletin). (Izvestiya of the Academy of Sciences of the USSR. Division of Chemical Science), 1989, 38(10), pp. 2140-2141, (in Russian). DOI: 10.1007/BF00962125.
35. Saratovskikh, E.A.; Orlov, V.S.; Krinichnyi, V.I. EPR spectroscopic study of metallocomplexes of 3,6-dichloropicolinic acid. Bulletin of the Academy of Sciences of the USSR, Division of Chemical Science (Russian Chemical Bulletin). (Izvestiya of the Academy of Sciences of the USSR. Division of Chemical Science), 1989, 38(11), pp. 2274-2277, (in Russian). DOI: 10.1007/BF01168068.
36. Saratovskikh, E.A.; Kondrateva, T.A.; Psikha, B.L.; Gvozdev, R.I.; Kartzev, V.G. Complex-formation of some pesticides with adenosine triphosphoric acid. Bulletin of the Academy of Sciences of the USSR, Division of Chemical Science (Russian Chemical Bulletin). (Izvestiya of the Academy of Sciences of the USSR. Division of Chemical Science), 1988, 37(1), pp. 2252-2258 (in Russian). DOI: 10.1007/BF00959872.
37. Saratovskikh, E.A.; Papina, R.I.; Kartsev, V.G. Binding of ATP by pesticides as a possible primary mechanism of inhibition of seed germination and plants growth. Agricultural Biology, 1990, 5, pp. 152-159, (in Russian).
38. Saratovskikh, E.A.; Papina, R.I.; Kondratieva, T.A. Violation of the energy metabolism of the cells in the process of seed germination of dicotyledonous and cereal plant under the action of different pesticides, and metal complexes. Cytology, 1999, 41(3-4), pp. 308-309, (in Russian).
39. Saratovskikh, E.A.; Kozlova, N.B.; Baikova, I.S.; Shtamm, E.V. Correlation between the toxic properties of contaminants and their contants of complexation with ATP. Russian Journal of Physical Chemistry B, Focus on Physics, 2008, 2(6), pp. 969-973. DOI: 10.1134/S1990793108060158.
40. Saratovskikh, E.A. Complexation with ATP as a Cause of Pollutants Toxicity to Aquatic Life. Journal of Environmental protection, 2013, 4(6), pp. 585-594. DOI: 10.4236/jep.2013.46068.
41. Blagoyi, Yu.P.; Galkin, V.L.; Gladchenko, G.O. Metal complexes of nucleic acids in solutions. Naukova dumka: Kiev, 1991, 272 p. (in Russian).
42. Saratovskikh, E.A.; Lichina, M.V.; Psikha, B.L.; Gvozdev, R.I. Character of the reaction of dinucleotides and polynucleotides with some pesticides. Bulletin of the Academy of Sciences of the USSR, Division of Chemical Science (Russian Chemical Bulletin). (Izvestiya of the Academy of Sciences of the USSR. Division of Chemical Science), 1989, 38(1), pp. 1822-1827, (in Russian). DOI: 10.1007/BF00957770.
43. Price, A.; Kelton J. Eds. Herbicides - Advances in Research. InTech: Rijeka, 2013, pp. 131-163. DOI: 10.5772/51496.
44. Luisi, P.L.; Baici, A.; Bonner, J.F.; Aboderin, A.A. Relation between fluorescence and conformation of ieNAD$^+$ bound to dehydrogenases. Biochemistry (ACS), 1975, 14(2), pp. 362-370. DOI: 10.1021/bi00673a024.
45. Saratovskikh, E.A.; Korshunova, L.A.; Roshchupkina, O.S.; Skurlatov Yu.I. Inhibition of NADH-oxidoreductases by metal compounds. Russian Journal of Physical Chemistry B. (Khimicheskaya Fizika), 2007, 26(8), pp. 46-53.
46. Tsuprun, V.L.; Akentreva, N.P.; Tagunova, I.V.; Orlova, E.V.; Grigoryan, A.N.; Gvozdev, R.I.; Kiselev, N.A. Electron microscopy methane-monoxygenase methane-oxidizing bacteria

Methylococcus capsulatus. Doklady Akademii nauk SSSR, 1987, 292(2), pp. 490-493 (in Russian).

47. Fitzpatrick, P.F.; Orville, A.M.; Nagpal, A.; Valley, M.P. Nitroalkane oxidase, a carbanion-forming flavoprotein homologous to acyl-CoA dehydrogenase. Archives of Biochemistry and Biophysics, 2005, 433, pp. 157-165. DOI: https://doi.org/10.1016/j.abb.2004.08.021.
48. Bayer, M.; Walter, K.; Simon, H. Purification and partial characterisation of a reversible artificial mediator accepting NADH oxidoreductase from *Clostridium thermoaceticum.* European Journal of Biochemistry, 1996, 239(3), pp. 686-691. DOI: 10.1111/j.1432-1033.1996.0686u.x.