Chapter

The Effect of Neonicotinoid Insecticides on the Structure and Stability of Bio-Macromolecules

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Abstract

Insecticides are among the most widely used pesticides in the world. They are preparations of chemical and biological origin used to control insects, which means its killing or preventing its destructive activity. Majority are used in forestry, agriculture, and households. Neonicotinoids represent the class of insecticides that is most frequently used in the world and replaced by more dangerous pyrethroids, organophosphates, and carbamates. In recent years, the focus has been mainly on the ecological and environmental risks caused by the use of neonicotinoids. These insecticides pose a very high risk to bees and also to soil and aquatic organisms. It is therefore highly topical to address the impact of neonicotinoids on biological systems on individual bio-macromolecules (DNA and serum albumins). Monitoring the impact of neonicotinoids on the structure and stability of biological macromolecules may contribute to reducing the use of these insecticides, as well as to considering and adjusting the tolerances of insecticides and their residues in food.

Keywords: insecticide, neonicotinoids, DNA, serum albumin, structure, stability

1. Introduction

The most widely applied pesticides in common practice are insecticides. They are preparations of chemical and biological origin used to control insects, which means their killing or preventing its destructive activity (**Table 1**).

Insecticides are mostly used in forestry, agriculture, and households. The use of insecticides has increased agricultural productivity, quantity, quality, and prolonged the lifetime of food and fodder plants. Insecticides have also revolutionized the fight against endemic diseases in developing countries. Unfortunately, these compounds pose the risk to humans and animals due to the presence of their residues in the food [4]. Insecticides are divided according to their mode of action into the ovicides (destroy eggs), larvicides (destroy larvae), and imagocides (destroy adult insects) [5]. The effect of insecticides is either immediate or slow acting and the insects die after a longer period of time [6].

1.1 Neonicotinoids

Neonicotinoids are a class of insecticides that belong to the most widely used in the world [7] because they allow for a rational approach to agricultural pest control [8, 9].

Insecticides class	Mechanism of action on pests	
Organochlorines	Dichlorodiphenyl-trichloroethane (DDT)	Destroying the delicate balance of Na^+ and K^+ ions in the axons of the neuron, which prevents the normal transmission of nerve impulses
	Hexachloro-cyclohexane (HCH)	Similar to DDT, only the effect is faster
	Cyclodienes	Act on the gamma-aminobutyric (GABA) acid receptor, which causes increased permeability of neurons to Cl^- ions
	Polychloroterpenes	Similar to cyclodienes
Organophosphates	Inhibition of nervous system enzymes, resulting in the accumulation of acetylcholine at neuron–neuron or neuron-muscle junctions	
Carbamates	Inhibition of aliesterase, which promotes hydrolysis of aliphatic ester bonds	
Formamidines	Inhibition of monoamine oxidase, which is responsible for the degradation of the neurotransmitters norepinephrine and serotonin	
Dinitrophenols	Inhibition of oxidative phosphorylation	
Pyrethroids	Influencing the peripheral and central nervous system	
Nicotinoids	Postsynaptic acetylcholine receptor blockade	
Neonicotinoids	Strong bond to nicotinic acetylcholine receptors in the central nervous system	

Table 1.
The major classes of insecticides and mechanism of action on pests [1–3].

Such insecticides tend to be referred to as "bio-rational" [10]. They replaced by more dangerous pyrethroids, organophosphates, and carbamates [11]. Discovery of imidacloprid and its subsequent introduction to the market in 1991 ushered in the era of neonicotinoids [12], which in 2014 accounted for more than 25% of the world's insecticide market [13]. We know the neonicotinoids of four generations. The first generation consists of chloropyridyls, which include imidacloprid, acetamiprid, thiacloprid, nitenpyram, cycloxaprid and paichongding. These are divided into three classes according to pharmacophore groups: N-nitroimine, N-cyanoimine, and nitromethylene [14]. Chlorothiazoles, as thiamethoxam, imidaclothiz and clothianidin, form the second-generation of neonicotinoids. Furanyls (e.g., dinotefuran) belong to the third generation. Fourth generation is made up of sulfoximines, such as sulfoxaflor [7, 15–18]. Neonicotinoids are used on all types of crops in temperate and tropical regions as well as in forestry, gardens, urban parks, and as veterinary control products ectoparasites in domestic animals [19]. The flexibility of their use is due to their system properties, which allow their application in the form of direct sprays on crops, soil granules or seed coating [20]. They have unique biological and chemical properties such as broad-spectrum insecticidal activity, low application rates and mode of action [21]. Due to their octanol/water partition coefficient and dissociation constant (pKa) values, they readily enter plant tissues and translocate to all parts of the plant regardless of the method of application. This will automatically become toxic to any insect and potentially other organisms feeding on plants [22, 23]. However, some of their characteristics increase their negative impact on the environment and non-target organisms [23, 24]. Recent studies show that neonicotinoids are already ubiquitous in the environment because of their versatile use, high mobility and relatively long half-life in water and soil [25, 26]. In the USA, for example, their presence was confirmed in twelve out of nineteen different

fruits monitored and vegetables, eleven of which contained more than two neonicotinoids. Value of thiamethoxam even exceeded the maximum residue limit. The most frequent occurrence was paradoxically found in foods primarily intended for infants and toddlers (prevalence ranging 6–31%) [27]. Neonicotinoid contamination has also been demonstrated in drinking water [28, 29], vegetables and fruit [30], milk [31], and in honey [32].

1.1.1 Mechanism of neonicotinoid action

Neonicotinoid's mode of action is by blocking the nicotinacetylcholine receptor (nAChR) leading to paralysis and death of the pests [33, 34]. NAChR is an ion channel responsible for immediate neurotransmission and belongs to the group of neurotransmitter ion channels along with gamma-aminobutyric acid, glycine, 5-HT3 and serotonin receptors. It consists of ten α, four β, γ, and δ subunits, which combine to form three basic types of receptors (muscle, neuronal, and ganglionic) with different structures. Different combinations of subunits result in differences in sensitivity to acetylcholine and other pharmacological systems. The most potent nAChR agonist is the nicotinic derivative epibatidine [35].

Neonicotinoids contain a negatively charged, electronegative cyano or nitro group that reacts with the positively charged nAChR site of the insect. In vertebrates, this interaction is blocked by the protonation of nitrogen in their organism [35].

1.1.2 Neonicotinoid toxicity

In recent years, the focus has been mainly on ecological and environmental risks caused by the use of neonicotinoids.

N-nitromine neonicotinoids show a very high risk to wild bees and honey bees [36]. Exposure to already low doses of insecticides occurs sublethal effects such as reduced immunity, disorientation and behavioral changes and reproduction of bees [37–40]. Based on these facts, the use of imidacloprid, thiamethoxam, clothianidin have been completely banned in the European Union since May 2018 [41].

N-cyanoimine insecticides in turn pose a high risk to soil and aquatic organisms [36, 42, 43]. Because of their long persistence in soil and high water solubility, they tend to pass into groundwater, surrounding rivers [44], lakes, and seas [45, 46]. Studies focusing on neonicotinoid content in watercourses have shown concentrations ranging from 0 to 380 ng/L depending on the region [47–49]. Because of adverse effect of neonicotinoids on aquatic organisms such as in food intake [50, 51], changes in swimming and nesting [52, 53], growth inhibition [54], changes in reproduction [53] and acute and chronic mortality [53, 55, 56], this contamination poses a major risk to aquatic ecosystem and for the supply water for its consumption [34, 57].

Several other studies of the adverse impact of neonicotinoids on non target organisms have demonstrated the development of immunosuppression in birds, bats, fish and amphibians [58]. Neonicotinoids can also adversely affect mammalian nAChRs, leading to neurobehavioral deficits and increased glial fibrillary acidic protein expression in the hippocampus [59–61], further affect the reproductive cycle, liver function and have genotoxic and neurological effects [11, 61–65].

Neonicotinoids are capable of disrupting the endocrine system. The study focused on exposure to thiacloprid in rats showed an increase in triiodothyronine and thyroxine hormones [66]. They are further associated with the development of oxidative stress [67], which in various cases leads to changes in the levels of ovarian

and antioxidant hormones [64], but also to increased germ cell apoptosis and DNA fragmentation in rat testes [68].

Next, we will discuss the most widely used representatives of first-generation neonicotinoid insecticides belonging to the group of N-cyanoimines (thiacloprid, acetamiprid) and N-nitroimines (imidacloprid).

1.1.3 Thiacloprid

Thiacloprid ((Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidine-2-ylidenecyanamide) is an insecticide belonging to the class of neonicotinoids (**Figure 1**), which is used for protection of vegetables, orchards, tea, maize and oilseed rape seeds [69].

Due to quite good solubility of thiacloprid in water and its low potential in groundwater, contamination of mainly surface water bodies of water, but its presence has also been detected in drinking water [28]. Thiacloprid occurs as a white to yellow powder [70] and it is polar, slightly soluble in water (0.185 g/L) and in organic solvents: dimethyl sulfoxide (150 g/L), acetone (64 g/L), ethyl acetate (9.4 g/L), acetonitrile (52 g/L). Partition coefficient octanol/water has a logP = 1.26 (pH = 7, 20°C), indicating poor solubility in fats, low absorption and distribution in the body and its pH is in the range of 4–9 stable [71].

In general, thiacloprid shows higher toxicity to aquatic organisms compared to other neonicotinoids studied [35]. This toxicity is probably related to its resistance to degradation in water at neutral and acidic pH values. WHO classifies it as moderately hazardous (Class II) [72] while the Environmental Protection Agency in the United States (US EPA) characterizes it as a potential carcinogen, based on the occurrence of thyroid tumors in male rats and uterine and ovarian tumors in rats, and mice [73]. Thiacloprid is metabolized and excreted in the urine within 24 hours after oral administration. The target organ is primarily the liver; a toxic effect on the liver has also been observed in dog prostate [41, 74]. Other studies have shown that it causes fetal 25 resorption, skeletal retardation, changes in motor activity in rats, thyroid adenomas, and uterine adenocarcinogens in mice [74]. In fish, after exposure to TCLs, there is growth retardation, delayed fetal development, and changes in antioxidant enzyme levels [75]. Moreover observed were reduced cell proliferation associated with higher levels of chromosomal aberrations in bovine lymphocytes [76] and genotoxic and cytotoxic effects on human [77] and bovine lymphocytes [78].

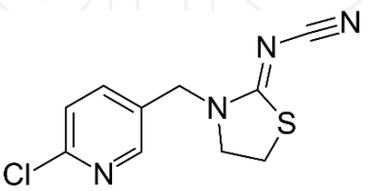

Figure 1.
Structural formula of thiacloprid.

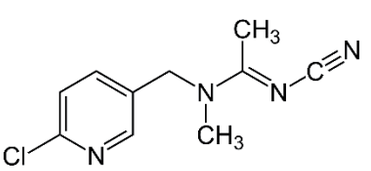

Figure 2.
Structural formula of acetamiprid.

1.1.4 Acetamiprid

Acetamiprid ((E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine) is a new broad-spectrum neonicotinoid (**Figure 2**) which is used to control sucking insects by interfering with insects'nervous system [79, 80]. It protects of crops such leafy vegetables, citrus fruits, pome fruits, grapes, cotton, cole crops, and ornamental plants. Acetamiprid plays a key role in commercial cherry farming due to its effectiveness against the larvae of the cherry fruit fly [20].

Acetamiprid occurs as a white crystalline substance, it is polar and water soluble (4.2 g/L), therefore it can be transported to surface waters and may be toxic to aquatic organisms and life. It is also soluble in organic solvents: acetone, chloroform and dichloromethane (200g/L) and no very frothy in hexane (0.005g/L). Partition coefficient octanol/water has a logP = 0.80 (pH = 7, 25°C) and its dissociation constant is pKa = 0.7 [81].

Acetamiprid contains a 6-chloro-pyridine motif in its molecule, as does the animal alkaloid epibatidine from the poisonous South American frog *Epipedobates tricolor* [82]. An important property of acetamiprid as a representative of N-cyanoimine neonicotinoids is that, unlike N-nitroimines such as clothianidin, dinotefuran, imidacloprid, thiamethoxam or nitenpyram, it is of little toxicity to bees [83]. It does not accumulate in soil, is mobile and rapidly degrades by aerobic mechanisms [9] and microorganisms are involved in its degradation [84]. Its half-life in soil ranges from <1 to 8.2 days [85] and its content in vegetables and fruits is low [86, 87]. The application of acetamiprid in greenhouses and agricultural farms is safe and not associated with major health risks [88]. Human poisonings are known only in cases where acetamiprid is used as a suicide agent. Two such cases of acute suicidal poisoning are described in the medical literature. In both cases, nausea and vomiting, muscle weakness, hypothermia, convulsions and other clinical manifestations, including tachycardia, hypotension, ECG changes, hypoxia and thirst, occurred. These symptoms are partly similar to acute organophosphate intoxication. Supportive treatment of the clinical symptoms was sufficient and both patients were discharged without complications 2 days after ingestion of acetamiprid [89]. It is not yet sufficiently clear whether acetamiprid is genotoxic to mammals [90].

1.1.5 Imidacloprid

Imidacloprid (N-{1-[6-chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl} nitramide) is a systemic insecticide (**Figure 3**) that acts as an insect neurotoxin, used for pest control in agriculture. In the year 1999, it was the most widely applied insecticide in the world [91]. Imidacloprid can be used by soil injection, broadcast foliar, application to the skin of the plant, tree injection, ground application as a liquid or granular formulation, or as a pesticide-coated seed treatment [92]. It is extra effective against sucking insects and mining pests such as mealybugs, aphids, thrips, and rice leafhoppers and against whitefly [93].

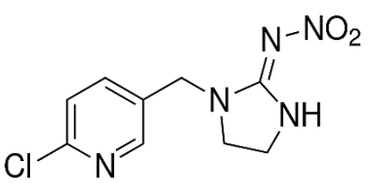

Figure 3.
Structural formula of imidacloprid.

Imidacloprid is a colorless crystalline substance with slight characteristic odor. It is weakly soluble in water (0.61 g/L) but better soluble in organic solvents: dichloromethane (67 g/L), isopropanol (23 g/L) and toluene (0.69 g/l). Partition coefficient octanol/water has a log P = 0.57 (pH = 7, 21°C) and its dissociation constants are pKa_1 = 1.56 and pKa_2 = 11.12 [94].

Imidacloprid has been classified by WHO as moderately hazardous (Class-II) based on animal studies [95]. The use of imidacloprid has a devastating impact on biodiversity, in particular on rivers and watercourses, not only on crustaceans [96], mollusks [97] and non-target species (insects), but also on soil organisms [36], as well as on bird populations [98]. Acute intoxication by imidacloprid and its metabolites resulted in the fast appearance of neurotoxicity symptoms, such as hyperactivity, hyperresponsiveness and trembling and led to hypoactivity and hyporesponsiveness [99]. It has been mentioned having harmful effects (oral toxicity) on honeybees in fields [100]. There is known case of imidacloprid poisoning with suicidal intent that developed various manifestations including hypokalaemia, central nervous system depression, respiratory arrest and paroxysmal atrial fibrillation [101]. Imidacloprid is metabolized by photodegradation from soil surface and water [102]. It is proven that plant metabolites of imidacloprid, the imidazolidine derivative, the olefin metabolite and nitroso-derivative were more toxic to aphids than imidacloprid itself [103].

2. Interaction of neonicotinoids with bio-macromolecules

Monitoring the interaction of neonicotinoid insecticides with biological macromolecules (DNA and serum albumins), determining the binding constant and the interaction mode provides a more comprehensive view of their distribution, toxicity and metabolism in the organism. It is also of great importance in adjusting the tolerance of these insecticides and their residues in food. The presented summarized results could contribute positively to the reduction of the use of the group of insecticides discussed by us.

2.1 Methods and analysis

The interaction of neonicotinoids with bio-macromolecules can be studied most often using UV–Vis, fluorescence and IR spectroscopy, circular dichroism, monitoring of bio-macromolecules melting, viscosity assays and last but not least molecular docking.

2.1.1 UV–Vis measurements

UV–Vis spectroscopy is a very effective method for investigating structural changes in the formation of DNA or protein and ligand/DNA or ligand/protein complexes. These measurements have been made on absorption spectrophotometer using quartz cuvettes of 1 cm path length at laboratory temperature. Usually the concentration of DNA or protein is constant while the concentration of neonicotinoid varies. A common practice is to add the same concentration of insecticide to the reference sample (with respect to the absorption maximum of the neonicotinoid). By monitoring the changes in absorbance intensity and the shift of the DNA/protein absorption maximum, it can be predicted how the neonicotinoid binds to the DNA/protein structure.

2.1.2 Fluorescence measurements

Fluorescence measurements were performed on spectrofluorimeter in a 1 cm quartz cuvette. Upon interaction, an extinguishing mechanism can be noted.

In general, extinguishing can be qualified as dynamic and static. The quenching mechanism is accurately described by the Stern-Volmer Eq. (1)

$$\frac{F_0}{F} = 1 + K_q \tau_0 = 1 + K_{SV}[Q] \tag{1}$$

where F_0 and F are the fluorescence intensities in the absence and in the presence of the quencher (Q) respectively. K_q and K_{SV} are the bio-macromolecule quenching rate constant, and Stern-Volmer constant respectively. τ_0 is the average lifetime of the bio-macromolecule without quencher and $[Q]$ is the concentration of quencher [104, 105].

Subsequently the binding parameters such as binding constant (K_a) and the number of binding sites (n) can be calculated according to Hill Eq. (2).

$$\log\left[\frac{F_0 - F}{F}\right] = \log K_a + n \log[Q] \tag{2}$$

The number of binding sites and association constant have been obtained by the plot of $\log[(F_0\text{-}F)/F]$ versus $\log[Q]$ [106–108].

2.1.3 Melting studies

The values of thermodynamic parameters can be determined from the Van't Hoff equation. This equation has been used in the form (3) for the interaction of neonicotinoid insecticides with proteins

$$\ln K_a = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \tag{3}$$

while ΔH is the enthalpy change, ΔS the entropy change, T is the temperature, and R is the universal gas constant. The binding studies from fluorescence measurements were usually realized at three different temperatures. By plotting $\ln K_a$ versus $1/T$ it can be possible to determine the values of ΔH and ΔS. Gibbs free energy change ΔG could be calculated using Eq. (4) [109, 110].

$$\Delta G = \Delta H - T\Delta S = -RT\, ln\, K_a \tag{4}$$

The thermal stability of DNA was studied by an absorption spectrophotometer with a Peltier module. A special type of Van't Hoff Eq. (5) has fitted melting curves

$$A = A_{\min} + \frac{A_{\max} - A_{\min}}{1 + e^{\left[\frac{\Delta H}{R} x \left(\frac{1}{T} - \frac{1}{T_m}\right)\right]}} \tag{5}$$

where A is the absorbance, $A_{\min}$, $A_{\max}$ are the minimal and maximal measured absorbance respectively, H is the enthalpy of transition, T, T_m are actual and melting temperature [111].

2.1.4 CD studies

CD spectroscopy was used to record changes in the secondary structure of optically active substances, including proteins and DNA. The CD results of complexes neonicotinoid/protein have been expressed in terms of mean residue ellipticity (MRE) (6)

$$MRE = \frac{Observed\ CD\left(mdeg\right)}{10\,c\,n\,l} \tag{6}$$

where c is the concentration of protein, n is the number of amino acid residues of protein, and l is the path length. The α-helix contents of free protein and neonicotinoid/protein complexes can be calculated from MRE values at 208 nm using the following Eq. (7) [112].

$$\alpha - helix\left(\%\right) = \frac{-MRE_{208nm} - 4{,}000}{33{,}000 - 4{,}000} x100 \tag{7}$$

CD spectra of DNA are very sensitive to the interaction mode of DNA with small molecules like neonicotinoid insecticides [113]. The characteristic spectrum of DNA in the B-form helix is characterized by a negative band at 245 nm resulting from right-handed helicity and a positive band at 278 nm resulting from base stacking [114]. By forming a neonicotinoid/DNA complex, a decrease in the intensity of the negative band at 245 nm was usually observed with accompanying red shift, while the intensity of the positive band at 276 nm increased. This increase in intensity was associated with a slight red shift. These changes of CD spectra represent the transformation from a B-form DNA structure to an A-conformation [115].

2.1.5 FT-IR spectra analysis

Infrared spectra have been collected approximately after 2h incubation of solutions containing neonicotinoid insecticides and bio-macromolecule at various rations. The amount of DNA or protein was constant. The intensity and shifting changes in infrared spectra could be applied to accurately specify the site of incorporation of neonicotinoids into the structure of bio-macromolecules (DNA and proteins) [116].

2.1.6 Viscosity tests

Viscosity tests can provide further information about the nature of the interaction neonicotinoid insecticides and DNA. This type of experiment consists in measuring the relative viscosity (t/t_0) of ethidium bromide/DNA and neonicotinoid/DNA complexes. A significant increase in viscosity was detected during the interaction of a typical intercalator ethidium bromide and DNA. The task was to compare the changes in DNA viscosity after adding neonicotinoids to the solution. If the same increase in viscosity was observed as for ethidium bromide, then the intercalation mode of binding between insecticides and DNA could be predicted [117].

2.1.7 Molecular docking studies

The trend of today's research is to confirm the results obtained from experiments by molecular docking studies. Serum albumin and DNA crystallographic

data can be used from the Brookhaven Protein Data Bank. The species selected were 1AO6 (HSA), 4F5S (BSA), and 453D (DNA) [116, 118]. The molecular structures of neonicotinoid insecticides could be taken from The PubChem database. Most often AutoDockVina has been used for molecular docking and molecular simulation of the interaction between neonicotinoids and bio-macromolecules [119].

2.2 Interaction of neonicotinoids with DNA

Neonicotinoid insecticides are highly reactive compounds that form complexes with a variety of cellular biomolecules, including DNA. DNA plays an important role in cell proliferation, protein synthesis and genetic transcription. It is necessary to protect DNA from the harmful effects of insecticides because they will damage the genetic structure of cells and disrupt metabolic processes [120]. The study of the interaction between insecticides and nucleic acids is considered to be important to allow screening for carcinogenic properties of pesticides, especially insecticides [121, 122]. Studies addressing the specific mode of interaction and binding sites of neonicotinoid insecticides with DNA are few. Several techniques have been used to study the binding properties between neonicotinoid insecticides and DNA, including UV–visible absorption, fluorescence, circular dichroism, Fourier transform infrared spectroscopy, coupled with DNA melting investigations and viscosity measurements in physiological buffer. To predict the possible binding site and binding mode was used the molecular docking study [120–122].

Neonicotinoid insecticides belong to small molecules known to bind to the double helix of DNA by two dominant modes, especially such as groove binding and intercalation. Groove binding presents docking the thin ribbon-like molecules in the DNA minor groove, in close proximity to the sugar-phosphate backbone. Conversely, their intercalation into the helix includes the insertion of a drug – usually a planar aromatic cation – into the base reservoir of the helix [123].

It has been demonstrated that thiacloprid interacts with DNA, which influences on the length and denaturation of DNA. The binding strength of thiacloprid and DNA is expressed by a binding constant K_a whose value is $9.3{\cdot}10^3$ L/mol at laboratory temperature [117]. The presence of DNA significantly affects the emission spectrum of thiacloprid. The quenching of its fluorescence intensity was analyzed using the Stern-Volmer method [114]. The quenching constant K_{SV} was determined to be $2.8{\cdot}10^4$ L/mol and correlation coefficient for K_{SV} was $R = 0.998$. Not considerable changes in viscosity behavior were observed as a result of its addition to the DNA solution. The presence of thiacloprid causes a decrease in melting temperature T_m and Van't Hoff enthalpy ΔH of DNA. These changes in thermodynamic parameters lead to destabilization of the DNA molecule. In addition, it was recorded a two-phase character melting curve of the complex DNA-thiacloprid with an expression destabilization of AT regions in DNA [117]. It is generally assumed small molecules that consist of at least two aromatic rings coupled by a no rigid bond enabling their torsional flexibility, like thiacloprid bind preferentially to the minor groove of DNA, specially to the regions rich in AT-base pairs [124, 125]. Considering all the above results, it can be concluded that thiacloprid does not interact with DNA via an intercalating binding mode. Increasing of the length and influencing of DNA denaturation points to the groove-binding mode of interaction. Probably incorporation of this insecticide occurs into DNA minor groove by hydrophobic or hydrogen bonds [117].

Similarly, the effect of acetamiprid on DNA structure and stability was investigated. As shown by the UV–visible spectra, after the addition of acetamiprid to DNA solution, the absorption peak of DNA at 260 nm (associated with strong purine and pyrimidine base absorption in DNA [126]) increased markedly and there was

significant blue shift. The fluorescence emission spectra of acetamiprid upon addition of DNA revealed that increasing concentration of DNA gradually decreased fluorescence emission peak of acetamiprid and a new band developed at approximately 370 nm. These facts can be attributed to the formation of the acetamiprid-DNA complex. The quenching mechanism between acetamiprid and DNA was analyzed by use of the Stern–Volmer method. The obtained K_{SV} value was $1.86 \cdot 10^4$ L/mol and the relevant correlation coefficient for K_{SV} was $R = 0.997$. The binding constant value K_a was determined $5.27 \cdot 10^3$ L/mol. Both constants were found at laboratory temperature [116]. Small molecules intercalating into the DNA causes stabilization of base stacking and leads to significant increase of *Tm,* whereas non-intercalation binding leads to no obvious increase in melting temperature [127]. In acetamiprid binding, T_m DNA increased by approximately 3°C. It can therefore be deduced that acetamiprid interacted with DNA via no classic rather than classical intercalation. This result was supported by evidence obtained from molecular docking, namely, the intercalation of acetamiprid into the double helix DNA from one side. Measurement of the relative viscosity of DNA shows a significant decrease in its viscosity, which excludes the classical intercalation mode of binding. The model in which acetamiprid binds to DNA in a partial intercalation manner explained this phenomenon. This means that partial intercalation can occur where acetamiprid can act as a 'wedge' that pushes apart one side of the base pair stack (but in contrast to the classical intercalation model, does not completely separate the stack), and thus induce static bending or kinking in the double helix [128]. Thermodynamic data for the interaction of acetamiprid and DNA were calculated. The Gibbs free energy value ΔG has a negative sign, indicating that the process is happening spontaneously [116]. Positive values of enthalpy ΔH and entropy ΔS changes suggest that hydrophobic interactions play an important function in the formation of the bond between acetamiprid and DNA and stabilize the complex [129]. Significant changes, in position and intensity, were observed in FTIR spectra for the GC base pair than for the AT base pair. It can be predicted that the specific binding site of acetamiprid to DNA is a site rich in GC base pairs. In addition, it is confirmed that acetamiprid binding leads to a change in the secondary structure of DNA from the B conformation to the A [116].

The interaction of acetamiprid with DNA is the basis for the development of the DNA probe, which is used in practice to detect the presence of acetamiprid in environmental samples and agricultural products. The results of the detection of acetamiprid using the DNA probe are in almost complete agreement with the results obtained using the HPLC technique [130].

At present, the interaction of imidacloprid with DNA is poorly studied, as its mode of incorporation into DNA, exact binding site, and binding constant are not known. Nevertheless, it is proven that imidacloprid can induce oxidative stress and DNA damage in zebrafish [131] and bees [132]. The study of the interaction between imidacloprid (also other neonicotinoid insecticides) and DNA is important for us to understand the insecticidal mechanism of neonicotinoids and their side effects, such as carcinogenesis, teratogenesis and mutagenesis [133]. Therefore, the mechanism of the reaction between imidacloprid and DNA is necessary to examine in detail.

2.3 Interaction of neonicotinoids with serum albumins

The binding study of neonicotinoids with proteins has toxicological importance [134]. The results of these interactions may cast some light on the future study of the interaction between neonicotinoid insecticides and other proteins such as enzymes and have toxicological to ecotoxicology importance. Therefore, it is essential to investigate the effect of neonicotinoids on the structural and optical properties of serum albumins, especially human serum albumin (HSA), the thermodynamic aspects in

the binding process, and characters of the binding sites. The binding of insecticides, but also pesticides in general, to proteins has been exploited in the construction of pesticide biosensors. Biosensor assays can bring measures of the toxic effects of chemicals on the target organism and of the molecular mechanisms that are the basis of toxicity [135]. Another important direction in studies of the biological properties of neonicotinoids is directly related to protection of the health of humans and agricultural animals, and includes study of the interactions between these compounds and proteins, enzymes, and receptors in blood plasma and tissues. The structure of neonicotinoid insecticides contains sections and groups capable of forming electrostatic, hydrophobic, and hydrogen bonds as well as other types of bonds typical of endogenous ligands in their complexes with proteins. It is the reason many insecticides can play the important role of exogenous ligands and change their own properties within the composition of protein complexes, as is true for natural bio-regulators such as hormones. These alternations can involve metabolic parameters and biological effects of the pesticides in the human and animal bodies. The mechanism for the interaction of neonicotinoid with serum albumin probably includes "recognition" and initial binding of the ligand because of its polar group. Followed by adaptation of the ligand-binding site of the HSA molecule for binding by means of conformational transitions. This mechanism is terminated by subsequent interaction of the hydrophobic core of the ligand with the nonpolar side chains of serum albumin in the cavity of the binding site. The new insights into the interaction of serum albumins and neonicotinoid insecticides, about the forms in which neonicotinoids exist in the body and the approaches developed for studying interactions of these compounds with the major transport protein HSA are a necessary basis for estimating the biological effects of pesticides in this class when they enter human blood [136].

It has been shown that thiacloprid interacts with HSA, its binding properties have been characterized at the molecular level under physiological conditions. The intensity of HSA absorption maximum decreased after the addition of thiacloprid, and its little red shift occurred at the same time. This indicated the probable formation of a complex between thiacloprid and HSA. With a gradual increase in thiacloprid concentration, a period decrease in fluorescence intensity was also observed. The Stern-Volmer plots were used for analyses of the quenching mechanism. The obtained K_{SV} value was $3.304 \cdot 10^4$ L/mol and the binding constant K_a was found $3.07 \cdot 10^4$ L/mol. Both constants were determined at laboratory temperature. The linear Van't Hoff equation was applied to track changes in the thermodynamic parameters. The entropy, enthalpy and Gibbs free energy values indicate that the coupling of thiacloprid to HSA is an exothermic process due to the positive value of ΔS and negative values of ΔH and ΔG [134]. It is well known that thermodynamic parameters play an important role in determining the type of binding by which a ligand (insecticide) binds to an HSA. Positive value of ΔH and ΔS is the result of hydrogen bonding. Negative values of these quantities (ΔH, ΔS) are corresponded with hydrogen bonds and van der Waals interaction in a low dielectric solution. The electrostatic interaction in aqueous solution between ionic species is associated with a positive change of ΔS and very small negative change of ΔH, almost zero [137]. Hydrophobic force and electrostatic force interactions are characterized by negative value of ΔH and positive ΔS values. In view of the above facts, it is not easy to interpret the results obtained from the thermodynamic analysis of the thiacloprid-HSA interaction. It is difficult to explain this interaction mode by a single intermolecular force. It has been published that the interaction force acting between small molecules and proteins is generally not just a single force. Probably may be there are variety of forces existing in the interaction forces which contain the electrostatic force. The hydrophobic molecule embedded in the internal hydrophobic region of proteins can be responsible for the fluorescence quenching [138, 139]. One

can hypothesize that the incorporation of thiacloprid into HSA can be realized by hydrophobic interaction, as evidenced by the positive ΔS value, but it cannot be excluded the influence of electrostatic interaction. The given conclusions are consistent with molecular modeling. This suggests that thiacloprid could be located on the surface of the binding pocket of subdomain IIA in the HSA molecule. It also confirms that the hydrogen bonding also plays a significant role [134]. Study of HSA secondary structure revealed the decrease of the percentage α-helix structure the effect of thiacloprid binding on the amino acid residue of the main polypeptide chain of HSA. Thiacloprid upset their hydrogen bonds [140].

Hemoglobin is essential protein in the blood plasma. It is working as a transporter of oxygen. Bovine hemoglobin (BHb) is used as a model protein to study the binding properties of drugs and insecticides [141]. The results obtained from the study of the influence of thiacloprid on this model protein show that their interaction is a static process. Two types of bonds play an important role in this binding, namely, hydrogen bonding and hydrophobic interactions. Increasing concentration of thiacloprid causes a marked decrease in BHb fluorescence intensity. The strength of cross-linking is characterized by the binding constant K_a, the magnitude of which was determined to be $8.04 \cdot 10^4$ L/mol at laboratory temperature. Tracking changes in thermodynamic parameters suggests that hydrogen bonding forces are most important for a given interaction. This is evident from the fact that negative values of the enthalpy change ΔH and entropy change ΔS were calculated. Changes in the secondary structure of BHb in the presence of thiacloprid show that there is a 3.7% decrease in α-helix content. Molecular modeling was used to determine the amino acid residues involved in thiacloprid-BHb binding. The thiacloprid pyridine ring interacts with Leu105, Pro95, Trp37 by hydrophobic interactions. The incorporation of thiaclopride into BHb is not exclusively hydrophobic, as several ionic and polar residues (Thr137, Tyr35) are present near the bound ligand. These polar residues stabilize the neonicotinoid insecticide via H-bonding and electrostatic interactions. Trp37 is involved in the formation of H-bonds with side chain imino groups [142].

Similarly, the effect of imidacloprid on HSA structure was investigated. The absorbance of the imidacloprid-HSA complex decreased with increasing imidacloprid concentration. A shift of the absorption maximum to the red region was also observed as in the thiacloprid interaction [135]. The HSA molecule contains 585 amino acid residues forming a single polypeptide of known sequence [118]. The protein response to conformational transitions, subunit association, ligand binding, or denaturation are changes in tryptophan emission spectra [143]. The study of the intrinsic fluorescence of Trp HSA is important for a better understanding of the specific changes that occur in the macromolecule [135]. Tyrosine fluorescence in HSA is mostly quenched due to the presence of nearby amino acids or efficient energy transfer from tyrosine fluorescence to Trp214 [144]. Imidacloprid caused a decrease in the fluorescence intensity of tryptophan residues. Fluorescence resonance energy transfer determined the distance between the donor (HSA) and acceptor (imidacloprid). Its size is 2.10 nm [135]. It is generally accepted that the average distances between the donor fluorophore and the acceptor fluorophore are 2–8 nm and indicate that energy transfer from HSA to imidacloprid occurs with a high probability [145]. It represents static quenching. During the measurement, a change in the structure of the surroundings of the Trp and Tyr residues was observed. Imidacloprid affected the physiological function of HSA. The fluorescence maximum of HSA shifted to higher wavelengths. The above results suggest that imidacloprid formed a specific bond in subdomain IIA, close to the tryptophan residue at position 214 of the HSA polypeptide chain like thiacloprid [135, 136]. The binding constant K_a was determined to the value $1.51 \cdot 10^4$ L/mol [135]. Thermodynamic parameters allowed predicting that

imidacloprid incorporates into HSA via hydrophobic interactions. These occur in the presence of an aromatic pyridine imidacloprid ring. Its nitroimine group could provide further enhancement of the affinity of the above neonicotinoid insecticide for HSA [135, 136].

A study looking at the interaction of imidacloprid with bovine serum albumin (BSA) gave the same results as in the interaction with HSA. From the fluorescence quenching spectra, the binding constant K_a was calculated, the magnitude of which reaches a value of $3.42 \cdot 10^4$ L/mol. Static quenching of emission without energy transfer by radiation within a single BSA molecule was also detected. Variations in thermodynamic parameters ($\Delta H > 0$, $\Delta G < 0$, $\Delta S > 0$) made it possible to predict the type of interaction. It is probably a hydrophobic interaction. Among the effects, that imidacloprid has on BSA can include increasing the polarity of the microenvironment in which Trp and Tyr are found, increasing the compaction of peptide bonds, and modifying the conformation of BSA [146].

The effect of acetamiprid on the structure of serum albumin is currently not sufficiently studied (not enough relevant results have been published). Considering the above results obtained by studying the interaction of similar neonicotinoid insecticides (thiacloprid and imidacloprid), it can be assumed that acetamiprid also affects the structure and conformation of serum albumins as well as their thermodynamic parameters. Therefore, it is essential to study and characterize in detail the incorporation of this insecticide into albumin.

3. Conclusion

In conclusion, we have to state that the topic discussed by us is still insufficiently studied and it is necessary to further continuously address the issue of the interaction of neonicotinoid insecticides with bio-macromolecules. Using of neonicotinoid insecticides leads to serious environmental problems, including contamination of soil and ground water, which can cause them to accumulate in the human and animal bodies and subsequently damage DNA. Several studies published in this chapter show that molecules of thiacloprid, acetamiprid, and imidacloprid are able to incorporate into important biological macromolecules and disturb their structure and function. All of them are honey bee killers and harmful to pollinators. Only thiacloprid is allowed to use in EU, imidacloprid and acetamiprid are prohibited which are considered to be one of the causes of bee colony decline in the world [41, 147]. On the other hand, they can still stay in the US market by Environmental Protection Agency decision [148].

However, the published results clearly indicate that neonicotinoid insecticides such as thiacloprid, acetamiprid, and imidacloprid, should be used very sparingly and cautiously in practice, especially in densely populated countries of the world where insecticides are overused.

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Conflict of interest

The authors declare no conflict of interest among themselves.

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