Scientific Paper

NMR Study of the Influence of pH on the Persistence of Some Neonicotinoids in Water[†]

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Abstract

The influence of the pH on the persistence of four neonicotinoid insecticides: imidacloprid, thiamethoxam, acetamiprid and thiacloprid, was studied by ^{1}H NMR spectrometry at different pH values of Britton-Robinson buffers (4.0, 7.0 and 9.0) at a constant temeprature. Thiamethoxam and imidacloprid, having an identical nitro-guanidine functional group, exhibited low persistence in alkaline media, as their degradation started already after three and five days, respectively. On the other hand, at pH 4.0 and 7.0 they remained unaffected for several months. In contrast to them, thiacloprid and acetamiprid, the compounds with a cyanoimine functional group, were less persistent in acidic solution. In alkaline media, they were stable for about 30 days. The proportion of hydrolyzed pesticide was calculated from the ^{1}H NMR spectra by comparing the integrals of compound signals with the signal of acetate standard.

Key words: imidacloprid, thiamethoxam, acetamiprid, thiacloprid, persistence, ^{1}H NMR-study.

Introduction

Neonicotinoids are a relatively new class of systemic insecticides with a distinct mode of action. Namely, the biological activity of neonicotinoids is ascribed to their interference with the nicotinic acetylcholine receptors and, therefore, they exhibit specific activity against the insect nervous system. This unique mode of action makes these compounds highly applicable for controlling the biological effect of insects in cases when these developed resistance to conventional organophosphate, carbamate and pyrethroid insecticides.[1-6] They systematically control a broad spectrum of chewing and sucking pests and represent developmental insecticides that are used worldwide in a variety of agricultural crops and seed treatment.[7-9] In European Union and in our country, the use of four neonicotinoids: acetamiprid ((1*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N'*-cyano-*N*-methylethanimidamide), thiacloprid ([3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]cyanamide), thiamethoxam (3-[(2-chloro-5-thiazolyl)methyl]tetra-hydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine) and imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-*N*-nitro-1*H*-imidazol-2-amine]) (Figure 1) have been approved by the year 2004.[3]

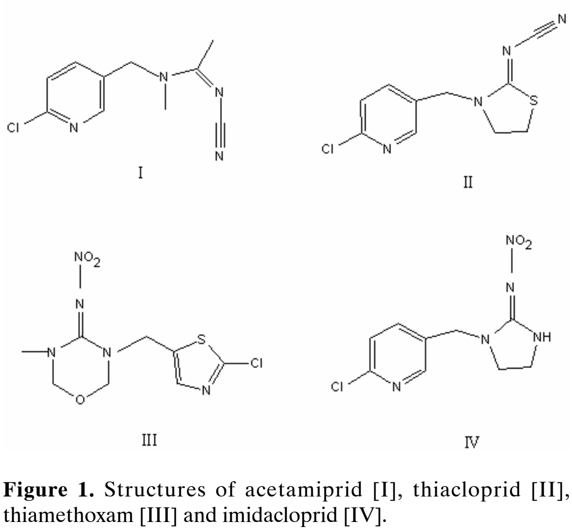

Figure 1. Structures of acetamiprid [I], thiacloprid [II], thiamethoxam [III] and imidacloprid [IV].

The persistence and hydrolysis of pesticides is an area that has received extensive study, since these compounds can enter the environment at some stage and dissolve in groundwater or adsorb in lipophilic media. It is also useful to understand the hydrolysis pathway in order to determine the stability of a particular pesticide, to identify its hydrolysis products, and thus assess its toxicity and analyze the residues.

As observed by Zheng and Liu[10], the persistence of imidacloprid is primarily determined by the pH and temperature. They found that imidacloprid is stable in acidic and neutral media, but hydrolyzes readily in alkaline media, yielding only one main reaction product, 1-[(6-chloro-3-piridinyl)methyl]-2-imidazolidone. Sarkar et al.[11] have investigated the effect of pH and type of formulation on the persistence of imidacloprid in water and found that the persistence of imidacloprid was influenced by the type of formulation, initial concentration, and pH. They reported that the degradation rate of the parent compound followed the first order kinetics, the rate being slowest at pH 9.0. It has also been reported that imidacloprid degraded rapidly in the presence of sunlight.[12] A progressive photocatalytic oxidation of imidacloprid in aqueous solution over TiO_2 was reported by Malato et al.[13] Shwartz et al.[14] have investigated the products of direct photolysis of thiamethoxam and concluded that this insecticide degraded significantly under photolytic conditions at pH 5.0. The degradation products in the first step were 4H-1,3,5-oxadiazin-4-one and 3-[(2-chloro-5-thiazolyl)methyl]-tetrahydro-5-methyl- and N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl. Krohn[6] reported that thiacloprid was stable in water between pH 5.0–9.0 for a relatively long period of time.

The common ways of determining these insecticides and their degradation products are either chromatographic analysis (HPLC and GC)[10-20] or preparative isolation of degradation product with MS and/or IR analysis.[14]

Our previous investigation[21] and a recent study[22] showed that NMR spectrometry can be successfully applied for the investigation of photocatalytic degradation pathway and kinetics of some pesticides such as fungicide metalaxyl[21] and herbicide mecoprop[22]. To the best of our knowledge, no publication dealing with the NMR spectrometric investigation of the persistence of neonicotinoid insecticides has appeared so far. On the other hand, it has been shown that water-suppressed ^{1}H NMR spectrometry can be applied for the investigation of hydrolysis rate of different drugs (neostigmine bromide, carbachol, and atropine).[23,24]

In view of the above, as a continuation of our study in the area of neonicotinoids[25,26], this contribution is concerned with the possibility of applying water-suppressed NMR technique to study the persistence and hydrolysis of the mentioned four neonicotinoids (imidacloprid, thiamethoxam, acetamiprid and thiacloprid) at different pHs.

Experimental

Apparatus

The NMR spectrometric measurements were performed on a Bruker AC–250 instrument with standard Bruker software. All measurements were carried out using regular 5 mm NMR tubes. All pH measurements were made using an Iskra combined glass-calomel electrode, on a previously calibrated Iskra pH–meter.

Reagents and solutions

All chemicals used were of the analytical reagent grade. Aqueous solutions were prepared in doubly distilled water. The reference standards were thiamethoxam (Riedel-de Haën, Germany), purity 99.9%; imidacloprid (Riedel-de Haën), purity 99.9%; acetamiprid (Dr Ehrenstorfer GmbH, Germany), purity 99.0%, and thiacloprid (Dr Ehrenstorfer GmbH), purity 99.5%. Solutions were prepared in D_2O and $CDCl_3$ (Merck, Germany), purity 99.8%. The chemical shifts are referred to tetramethylsilane (TMS, δ_H = 0.00 ppm) in $CDCl_3$ and sodium salts of 3-trimethylsilyl-1-propane sulfonic acid (DSS, δ_H = 0.00 ppm) in D_2O.

Britton-Robinson buffer solutions were prepared from 0.04 M stock solutions of phosphoric (Merck), boric (Merck) and acetic (Merck) acids by adding 0.2 M solution of sodium hydroxide (Merck) to obtain the required pH value (4.0, 7.0 and 9.0).

The aqueous stock solutions were prepared by dissolving the insecticides in a mixture of the appropriate Britton-Robinson buffer solution and D_2O (1:1), to obtain saturated solutions at room temperature (23 °C).

The blank was a mixture of the appropriate Britton-Robinson buffer solution and D_2O (1:1).

Hydrolysis measurement

To study the persistence and hydrolysis rate of neonicotinoids all glassware were heat sterilized, and solutions were prepared in doubly distilled water. The solutions were kept in regular 5-mm airtight NMR tubes and stored at 23 °C. To ensure the insolation conditions similar to the natural ones the samples were kept in the natural day-night regime for four months.

Other experimental conditions: temperature of NMR samples was 23 °C, relaxation delay 0.5 s, data points 32 K, time domain 32 K, receiver gain 1, number of dummy scans 2, pulse width 2.0 μs, acquisition time 3.28 s, spectral width 5000 Hz, line broadening 0.2. The numbers of scans were 32, 64, 128 and 256 for thiamethoxam, acetamiprid, imidacloprid and thiacloprid, respectively.

The most prominent features of the ^{1}H NMR spectra of parent compounds obtained in $CDCl_3$,

[δ_H (ppm)] are: thiacloprid [3.39 (*t*, *J* 7.5 Hz, 2H, H-5, Thia.), 3.79 (*t*, *J* 7.5 Hz, 2H, H-4, Thia.), 4.62 (*s*, 2H, CH_2), 7.37 (*d*, *J* 8.2 Hz, 1H, H-5), 7.67 (*dd*, J_1 8.2 Hz, J_2 2.5 Hz, 1H, H-4), 8.31 (*d*, *J* 2.5 Hz, 1H, H-2)]; acetamiprid [2.46 (*s*, 3H, $C(CH_3)$), 3.10 (*s*, 3H, $N(CH_3)$), 4.71 (s, 2H, $N(CH_2)$), 7.33 (*d*, *J* 7.5 Hz, 1H, H-5), 7.69 (*dd*, J_1 8.2 Hz, J_2 2.5 Hz, 1H, H-4), 8.31 (*d*, *J* 2.5 Hz, 1H, H-2)]; thiamethoxam [3.05 (*s*, 3H, $N(CH_3)$), 4.75 (*s*, 2H, $N(CH_2)$), 4.88 (*s*, 4H, H-2, H-6), 7.48 (*s*, 1H, H-4, Thia.) and imidacloprid [3.53 (*t*, *J* 7.5 Hz, 2H, H-4, Imid.), 3.81 (*t*, *J* 7.5 Hz, 2H, H-5, Imid.), 4.55 (*s*, 2H, $N(CH_2)$), 7.36 (*d*, *J* 8.2 Hz, 1H, H-5), 7.71 (*dd*, J_1 7.5 Hz, J_2 2.5 Hz, 1H, H-4), 8.20 (*s*, 1H, NH, Imid.), 8.33 (*d*, *J* 2.5 Hz, 1H, H-2)].

The persistence and hydrolysis were monitored by determining the rate of decrease of the characteristic signals in proton spectra. The degree of hydrolysis was calculated by comparing the integrals of NMR signals for the parent neonicotinoid molecule and those of the formed degradation products with the signal of acetate standard.

Results and discussion

Optimization of the measurement conditions

Figure 2 shows the ^{1}H NMR spectra of acetamiprid recorded for its solution in $CDCl_3$ (a) and D_2O (b). The obtained signals suggest the presence of two configuration isomers, one of which was thermodynamically more stable. Namely, in $CDCl_3$, four methyl signals are present from 2.46 to 3.10 ppm, of which two smaller singlets make ca. 20% of the two intensive singlets. In addition to the main component signals from the aromatic ring (δ range 7.33–8.31 ppm), there are other signals that belong to a minor component, whereby the signal at 7.69 ppm cannot be observed, probably because of the overlapping (Figure 2a). Data for the main component are already given (see above) and the chemical shifts for the minor component are [δ_H(ppm)]: [2.47 (*s*, 3H, $C(CH_3)$), 3.09 (*s*, 3H, $N(CH_3)$), 4.62 (s, 2H, $N(CH_2)$), 7.42 (*d*, *J* 4 Hz, 1H, H-5), 8.26 (*s*, 1H, H-2)]. Similarly, in D_2O (Figure 2b), the minor component, with the chemical shifts of 2.43 ppm and 3.00 ppm, makes 25% of the main component. Three proton signals from aromatic ring (δ range 7.42–8.26 ppm), are overlapped by the signals of the main component. This observation is also supported by the HPLC measurement. Namely, although the acetamiprid sample was an HPLC grade analytical standard, its purity checking by the chromatographic procedure recommended by the producer showed the absence of impurities. Hence, the observed presence of two components (main and minor) can be ascribed to the existence of two isomeric forms of acetamiprid. This phenomenon was not observed in the cases of the other three investigated neonicotinoids.

The NMR spectra in $CDCl_3$ could be taken more easily because of the higher solubility in this solvent, especially of imidacloprid and thiacloprid, which are in aqueous media about 10 times less soluble compared to the other two neonicotinoids. Another advantage of this solvent was the absence of signal overlapping around 4.8 ppm. Although $CDCl_3$ as solvent appeared to be advantageous, the mixture of D_2O-buffered water (1:1) was still chosen as it provided more natural conditions. Namely, the aqueous medium enables direct monitoring of the stability of neonicotinoids without additional sample preparation. Because the use of D_2O in the standard ^{1}H NMR measurements has certain disadvantages (a very intensive and broad HOD signal around 4.8 ppm), the water-suppressed technique was applied, whereby the signals from 4.6 to 5.0 ppm (usually CH_2 protons) were occasionally overlapped with residual water signal.

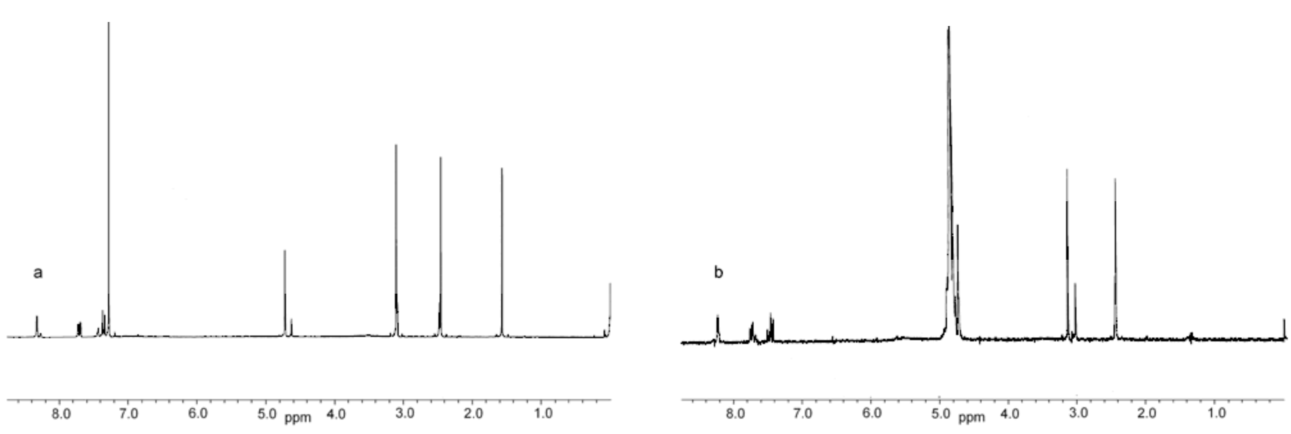

Figure 2. NMR spectra of acetamiprid in: a) $CDCl_3$ and b) D_2O.

The blank solutions containing Britton-Robinson buffers gave only one sharp methyl singlet at about 1.98 ppm, and it remained unchanged during the experiment. It should be noted that the spectra of the D_2O-buffered water (1:1) blank contained artifacts that could not be phased and were also present at an increased number of scans. This phenomenon was more pronounced at pH 9.0 (Figures 3c and 5) and it was observed at the highest concentration of NaOH in the solution. Similar artifacts were not present in the spectrum of pure D_2O. The useful signals could be easily distinguished because of their proper and phaseable shape.

Effect of pH on persistence and hydrolysis

Figure 3 shows the ^{1}H NMR spectral profiles of thiamethoxam taken in the course of the hydrolysis at pH 4.0 (a), 7.0 (b) and 9.0 (c). Thiamethoxam was the most stable at pH 4.0, exhibiting no significant changes in the spectra for the next four months. At pH 7.0, there were no significant changes in the spectra for five weeks, when the first detectable changes were registered. The intensity of the parent signal of aromatic ring at 7.60 ppm showed a very slow decrease, as well as of the two singlet signals from the six-membered heterocyclic ring at 5.02 and 5.10 ppm. In the spectrum recorded after 77 days new signals were observed, belonging to the hydrolysis intermediates. Thus, thiamethoxam hydrolyzed very slowly in acidic and moderately in neutral aqueous solutions; only about 3% of the initial amount decomposed during three months at 7.0. However, it was not the case at pH 9.0, as the hydrolysis was much faster in basic solutions. Namely, it appeared (Figure 3c) that the changes in thiamethoxam molecule at pH 9.0 occurred as early as after three days of experiment, as evidenced by the decrease of characteristic proton signals of the parent compound at 3.00; 5.02; 5.10 and 7.60 ppm. At the same time, one major product appeared in the spectra with the signals at 2.60, 4.52, 4.57 and 7.53 ppm.

At pH 9.0, the time-dependent decrease of proton signal at 5.10 ppm is evident from Figure 3c and Figure 4, curve 1. The data for the hydrolysis in alkaline solutions fit first-order kinetic equations. Also, it can be seen that after 15 days the rate of hydrolysis decreased as the hydrolysis time increased. The NMR pattern of aqueous thiamethoxam solution showed the presence of one major product (Figure 3c and Figure 4, curve 2).

Imidacloprid, having an identical nitro-guanidine functional group as thiamethoxam, exhibited a similar behavior. However, imidacloprid was more persistent in alkaline media, as its degradation started after five days. It also showed persistence at pH 4.0 and 7.0. We suppose that there is only one main hydrolysis product of imidacloprid in alkaline media [δ_H (ppm): 3.56, 3.64,

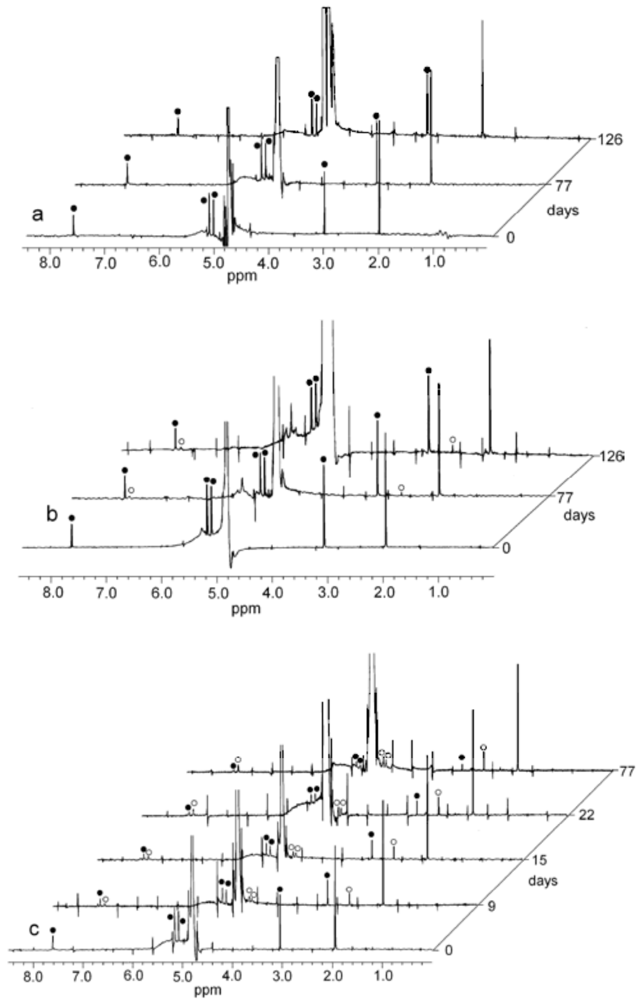

Figure 3. Temporal water-suppressed ^{1}H NMR spectral profiles of thiamethoxam at different pHs: a) 4.0; b) 7.0 and c) 9.0. ● parent compound, ○ new signals.

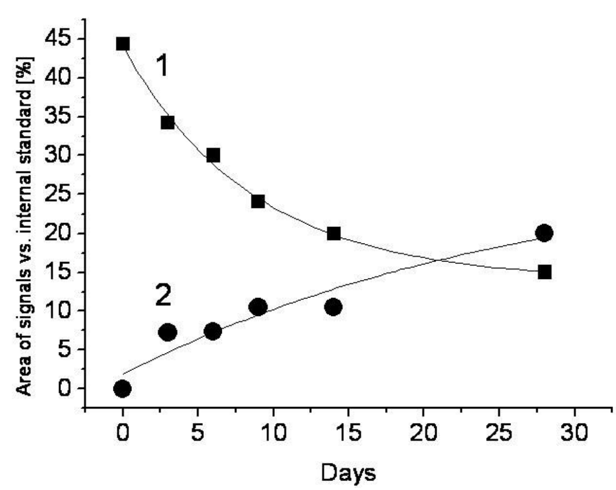

Figure 4. Time-dependence of the signal of the six-membered saturated-ring H during thiamethoxam hydrolysis at pH 9.0: 1) decrease of the parent compound at 5.10 ppm and 2) increase of the product at 4.57 ppm.

4.45, 7.51, 7.80, 8.15, 8.27] which agrees with previous observations.[10]

Having in mind the nature of the two considered compounds (imidacloprid and thiamethoxam), the rate of their disappearance and the rate of increase of the new signals, as well as the literature data,[10,13] we can propose a possible hydrolytic course. Namely, the strong electron-withdrawing NO_2 group increases the electrophilicity of the azomethine carbon of nitroguanidine functional group, so it is readily attacked by the nucleophilic OH^-.

Acetamiprid, the compound with the cyanoimine functional group, was less persistent in acidic Britton-Robinson buffer solution than the above two compounds. On the other hand, at pH 9.0 (Figure 5), first significant changes appeared after 30 days.

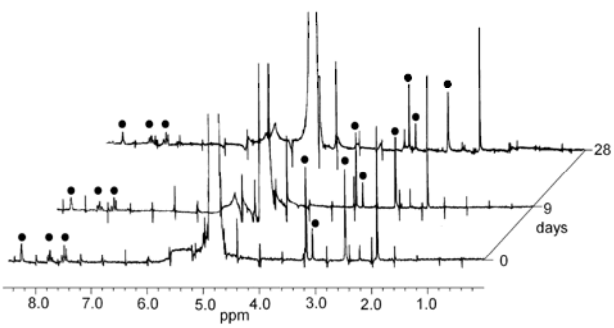

Figure 5. Temporal water-suppressed ^{1}H-NMR spectral profiles of acetamiprid in D_2O-Britton-Robinson buffer mixture at pH 9.0.

Because of the low solubility of thiacloprid in water (0.185 g/dm^3, 20 °C), study of its persistence in Britton-Robinson buffers by ^{1}H NMR spectrometry was possible but much less accurate. To enhance the method sensitivity and accuracy we extracted the species from a buffered water solution with dichloromethane, evaporated the solvent at 23 °C and dissolved the dry residue in $CDCl_3$. Figure 6 shows the ^{1}H NMR spectrum of the thiacloprid residue after 60 days at pH 9.0, obtained after applying such enrichment method. As can be seen, the parent compound was still present in measurable concentrations [δ_H (ppm): 3.39 (*t*, *J* 7.5 Hz, 2H, H-5, Thia.), 3.79 (*t*, *J* 7.5 Hz, 2H, H-4, Thia.), 4.62 (*s*, 2H, CH_2), 7.37 (*d*, *J* 8.2 Hz, 1H, H-5), 7.67 (*dd*, J_1 8.2 Hz, J_2 2.5 Hz, 1H, H-4), 8.31 (*d*, *J* 2.5 Hz, 1H, H-2)].

Generally, in the cases of imidacloprid, thiamethoxam and thiacloprid, the proton signals from the same substituted heterocyclic aromatic ring remained unchanged or were partly converted to similar signals, shifted upfield or downfield from the parent signals. Thus, it can be concluded that the hydrolysis under the given conditions did not affect the aromatic ring. Furthermore, all investigated molecules have

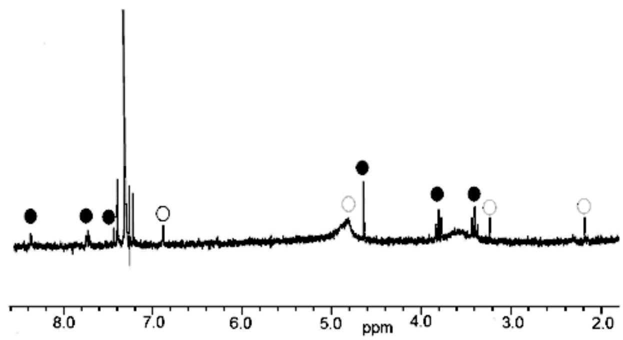

Figure 6. NMR spectrum of thiacloprid in $CDCl_3$, recorded after two months for enriched sample obtained at pH 9.0. ● parent compound, ○ new signals.

electron-withdrawing groups, either nitro-guanidine or cyanoimine, and thus they have carbon centers that are prone to the nucleophilic attack. The rate and pathway of hydrolysis of the investigated compounds are a function of the pH and nature of the attached functional groups in their molecules - saturated heterocyclic rings in thiacloprid, thiamethoxam and imidacloprid, and alicyclic molecular fragment in acetamiprid.

Conclusions

The presented water-suppressed ^{1}H NMR spectrometric study of the four neonicotinoids (imidacloprid, thiamethoxam, acetamiprid and thiacloprid) showed that their hydrolysis in the mixture of D_2O and Britton-Robinson buffers (pH 4.0, 7.0 and 9.0) is essentially dependent of the pH of the medium. As all investigated molecules have electron-withdrawing groups, the difference in the rate and pathway of hydrolysis are additionally determined by the nature of the functional groups in the vicinity of the carbon center that can be a target for the nucleophilic attack. The lowest persistence showed thiamethoxam at pH 9.0.

This study demonstrated that the applied NMR technique of recording time-dependent spectra can be successfully used to monitor the persistence of the investigated neonicotinoids and provide qualitative and quantitative information about their degradation products. The obtained results can serve as a basis for the development of other analytical methods for the determination of these pesticides.

Acknowledgement

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Povzetek

S ^{1}H NMR spektrometrijo smo raziskovali stabilnost štirih neonikotinoidnih insekticidov (imidakloprid, tiametoksam, acetamiprid in tiakloprid) pri različnih vrednostih pH Britton-Robinsonovih tamponskih raztopin (pH 4.0, 7.0 in 9.0). Tiametkosam in imidakloprid, ki vsebujeta nitrogvanidinsko funkcionalno skupino, sta slabo obstojna v alkalnem, saj začneta razpadati že po treh oziroma petih dneh. Pri pH 4.0 in 7.0 pa sta stabilna več mesecev. Nasprotno pa sta tiakloprid in acetamiprid, spojini s cianoiminsko funkcionalno skupino, manj stabilna v kislih raztopinah, a obstojna v alkalnem do 30 dni. Delež hidroliziranega pesticida smo izračunali iz ^{1}H NMR spektrov s primerjavo integralov signalov spojin in acetatnega standarda.