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Kinetic Evaluation of Imidacloprid Degradation in Mice Organs Treated with Olive Oil Polyphenols Extract

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Imidacloprid is a highly effective insecticide, acting as agonists at the insect nicotinic acetylcholine receptor. Nevertheless, imidacloprid itself or its metabolites could exhibit toxicity in mammals. Imidacloprid biotransformation involves oxidative cleavage, releasing the 6-chloronicotinic acid. Therefore, the concentration of imidacloprid and 6-chloronicotinic acid was used to characterize degradation kinetics and distribution of imidacloprid in mice liver, kidneys and lungs. Additionally, the influence of olive oil polyphenols on imidacloprid metabolism was evaluated. Experimental animals were divided into three groups: control, IMI – imidacloprid treated mice (5 mg/kg) and (IMI + PP) – mice treated with polyphenols (10 mg/kg) for seven days before the administration of imidacloprid. Neither imidacloprid nor 6-chloronicotinic acid could be detected 48 hours after administration in IMI group, while complete degradation in the (IMI + PP) group was accomplished within 24 hours. Significantly higher rate constant and shorter half-life in (IMI + PP) group emphasize that polyphenols may diminish the toxicity of this pesticide.

Keywords
imidacloprid
6-chloronicotinic acid
degradation kinetics
polyphenols
olive oil

INTRODUCTION

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine, IMI) is a new and potent nitromethylene insecticide with low soil persistence and high insecticidal activity at very low application rates. The favourable selective toxicity of imidacloprid to insects *versus* mammals is attributed to differences in their binding affinity in the nicotinic acetylcholine receptor (nAChR), making it safer for insect control than other neurotoxins (particularly organophosphates)[1] and enabling its diverse use for soil, seed, and foliar treatment in different crops.[2]

Studies on mammals suggest that IMI is rapidly and completely absorbed,[3] with 6-CNA and olefin as the main IMI's breakdown products (Figure 1).[4] Nevertheless, its use might be controversial, while some neonicotinoids or their metabolites may also up-regulate nAChR expression in mammals, and therefore could exhibit toxicity in mammalian hosts,[1] as indicated in human suicide case studies.[5,6] Also, the studies on insects have revealed several metabolites involved in IMI's toxicity, in particular olefine metabolite,[7] which is more toxic than IMI itself,[8] and 5-hydroxyimidacloprid.[9] Furthermore, the desnitro metabolite, has very little nervous system toxicity to insects[8] but is more toxic than IMI itself to mammals' nervous systems.[10] The soil metabolite 2-imidazolidone[11] (also known as ethyleneurea) induces tumors in combination with nitrate[12] and causes genetic damage.[13] These metabolites could act either alone or synergistically with

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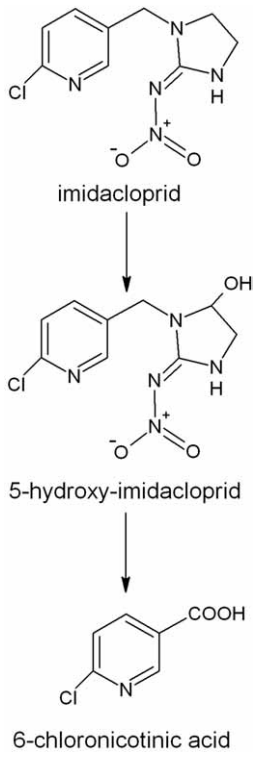

Figure 1. Oxidative cleavage of imidacloprid.

residues of the parent compound. Accordingly, kinetic evaluations are essential aspects in understanding the distribution of neonicotinoids and their metabolites throughout the body, as well as their toxity.

It is known that different classes of pesticides induce oxidative stress which may contribute to the toxicity of these xenobiotics.[14] Oxidative stress induction involves an excessive production of reactive oxygen species (ROS), resulting from impaired balance between the ROS generation and antioxidant defence capability. Collective actions of endogenous enzymatic and non-enzymatic antioxidants, comprising antioxidant defence mechanism, can be reinforced by dietary antioxidant intake, such as vitamins A, C and E.[15,16] One of the emerging strategies in modern toxicological sciences is the modulation of xenobiotic toxicity by nutrition.[17] Therefore, dietary intake of antioxidants might be a possible way to augment endogenous oxidative defence and subsequently modulate ROS mediated toxicity.

Recently, attention has focused on a vide variety of phenolic compounds, found in fruits, vegetables, wine, tea, cocoa and their products, as well as in olives and olive oil. These plant secondary metabolites are nonessential, non-vitamin dietary components, belonging to a group of phytochemicals,[18] with a wide range of biological effects.[19] Biological actions of polyphenols are mainly attributed to their free radical scavenging and antioxidant activity,[20] but in recent years, considerable attention has been paid to their abilities to induce detoxification defence system.[21,22]

As far as we know, there are insufficient data on factors contributing to degradation kinetics and the elimination of IMI and its metabolites from different hosts. As previously mentioned, the environmental health perspectives underlie the need for designing intervention strategies that modulate the toxicity of xenobiotics. Accordingly, the aim of this study was to evaluate the effect of treatment with polyphenols extracted from olive oil on the distribution and degradation rates of IMI in mouse liver, lungs and kidneys.

EXPERIMENTAL

Chemicals and Reagents

Methyl sulfoxide (DMSO), methyl cellulose (MC) and 6-CNA (99 %) were purchased from Acros-Organics (USA), while IMI (99.9 %) was from Riedel-de Haën (Germany). Each standard compound was dissolved in acetonitrile (1 mg/mL) and stored at 4 °C. Acetonitrile (HPLC-grade) and methanol (*p.a.*) were provided from J. T. Baker (Holland). Dichlormethane and n-hexane (*p.a.*) were from Carlo Erba (Italy).

Animals

Mice of BALB/c strains, aged 2–3 months, weighing between 25 and 30 g were used in the experiment. The animals were housed in groups of 5–6 in plastic cages, fed standard pellet food and given tap water *ad libitum.* Mice were maintained under a 12 hours dark-light cycle in a temperature ((20 ± 1) °C) and humidity ((50 ± 5) %) controlled facility.

Preparation of Pesticide Dose

Safe pesticide dose was derived from LD_{50}, determined on the basis of a pilot study, whereat IMI was diluted with DMSO and MC in order to obtain an effective mass ratio of 5 mg per kg of body mass.

Preparation of Olive Oil Polyphenols Extract

Polyphenols (PP) were extracted from 50 g of olive oil dissolved in hexane (50 mL) three times with 30 mL of a methanol-water mixture (vol. ratio 60:40).[23] In a respective hydroalcoholic extract, the total polyphenol content was determined spectrophotometrically (Cary 100 Bio, Varian) according to the method described by Gutfinger.[24] The total polyphenol content was 64.72 mg/kg expressed as caffeic acid.

Hydroalcoholic extract was evaporated to dryness at 40 °C using rotary evaporator, and the dry residue was resuspended in a 0.9 % solution of NaCl. This polyphenol extract was given to the experimental animals in a dose of 10 mg/kg body mass.

Treatment of Animals

Experimental animals were assigned in three groups *i.e.* control, IMI and (IMI + PP) group, and kept on a standard diet for 7 days. On the 7th day the control group received

vehicle alone (DMSO + MC), while the IMI group received pesticide administered intraperitoneally in a single dose of 5 mg/kg. A dose of 10 mg/kg of the polyphenols extract was intraperitoneally injected daily on 7 successive days, followed by IMI administration 2 hours after the last polyphenols dose ((IMI + PP) group).

Tissue Samples Preparation

After 7 days, the animals were sacrificed by cervical dislocation 15 min, 1, 6, 12, 24 and 48 h after the treatment with IMI or vehicle alone. The liver, kidneys and lungs were removed and placed in 0.9 % NaCl solution. Homogenates of tissue samples were prepared by adding 0.3–0.5 g tissue to 2 mL distilled water. The liquid-liquid extraction was performed three times with 5 mL dichloromethane, and the tubes were agitated on a platform shaker for 15 min. After centrifugation at 2000 *g* for 10 min the combined supernatant was evaporated to dryness at 40 °C using a rotary evaporator. The dried extracts were reconstituted with 500 μL of mobile phase and an aliquot (20 μL) was injected into the HPLC system.

Analysis of Imidacloprid and 6-CNA by HPLC

The residues of IMI and its metabolite 6-CNA were estimated by HPLC using a TSP Spectra System equipped with a UV/Vis detector. A Supelco reverse phase C_{18} (cartridge) column was used (150 mm length, 46 mm ID). The mobile phase, composed of acetonitrile and water, (vol. ratio 20:80) was applied at a flow rate of 0.8 mL/min. Column temperature was held at 25 °C. The retention times of IMI and 6-CNA were 4.3 and 1.6 min, respectively.

Evaluation of Imidacloprid Degradation Kinetics

To measure degradation rates in mice organs the decline of the IMI concentration as a function of time was monitored. For such degradation reactions it can be assumed that the probability of a molecule being chemically transformed remains constant and is independent of its concentration. This implies that the reaction rate declines proportionally to the concentration as a function of time (*i.e.* first-order kinetics). In this case the concentration follows an exponential decline curve according to the equation:

$$c = c_0 \cdot e^{-k_t \cdot t} \quad (1)$$

where c_0 and c represent initial and residual IMI concentrations, while k_t is the rate constant. The k_t can be expressed by means of the half-life using equation:

$$k_t = \frac{\ln 2}{t_{1/2}} \quad (2)$$

The half-life is the time after which half of the original amount of substance present has been chemically transformed, while so called $t_{1/2}$ is the time-interval after which the half of the originally present substance has disappeared. Rate constant (k_t) was determined by the integral method according to Gomzi.[25]

Statistical Analysis

The data were analyzed using Software Statistica, Version 7.0. Statistical significance was calculated by Mann-Whitney U-test. Data are reported as mean ± SD. The results were considered statistically significant at $P<0.01$ or $P<0.05$.

RESULTS AND DISCUSSION

Kinetic evaluations play an essential role in quantifying possible toxicity of a pesticide and its metabolites. A kinetic model then forms the basis for the prediction of the pesticide concentration time course under physiological and pathophysiological conditions.

Results from studies with rats and mice indicate that there are two major routes by which the IMI molecule is metabolized.[3,26] The predominant metabolic pathway involves oxidative cleavage, releasing the pyridinyl moiety as 6-CNA. The mass fraction (w) of IMI and 6-CNA was used to characterize degradation kinetics and distribution of IMI in mice liver, kidneys and lungs. Additionally, the influence of polyphenols extracted from olive oil on the degradation rate of IMI was evaluated. The IMI mass fraction found in the liver, kidneys and lungs 15 min after the administration was considered as the initial level, IL (100 %), and at the same time represents the maximum w(IMI) obtained. Residual w(6-CNA) was also expressed in respect to the IMI initial level. Regarding the IL, IMI rapidly decreased with time in the IMI and (IMI + PP) group in all tested organs.

A decrement of IMI, observed in the IMI group, 4 hours after the administration in the liver (25 % IL) is consistent with the results by Ford and Casida (27 % IL).[27] After 24 hours, w(IMI) decreased further to the value of 0.037 % IL. In the kidneys and lungs 6 h after the IMI administration, w(IMI) decreased to 35 % and 30 % IL, respectively. In these two organs, IMI could not be detected after 48 hours.

Treatment with an olive oil polyphenols extract in the (IMI + PP) group displayed a different distribution of IMI in the tested organs. The initial liver level decreased after 6 h to 4.8 % and after 12 hours IMI could not be detected. A statistically significant decrease of the IMI fraction was also detected in the lungs (10.8 %) and the kidneys (11.3 %) after 6 hours (Figure 2).

Simultaneously, the highest value of w(6-CNA) in these organs 6 hours after the IMI administration in the IMI group, was found to be 24 % in the liver, 56 % in the kidneys, and 48 % in the lungs with respect to the initial IMI level.

After the treatment with an olive oil polyphenols extract in the (IMI + PP) group, 6-CNA peaked much earlier in the liver and lungs *i.e.* one hour after the administration (30 % *vs*. 58 % IL), while in the kidneys the highest level was detected after 6 hours (90 % IL), as shown in Figure 3. This high kidney level indicates a quick pas-

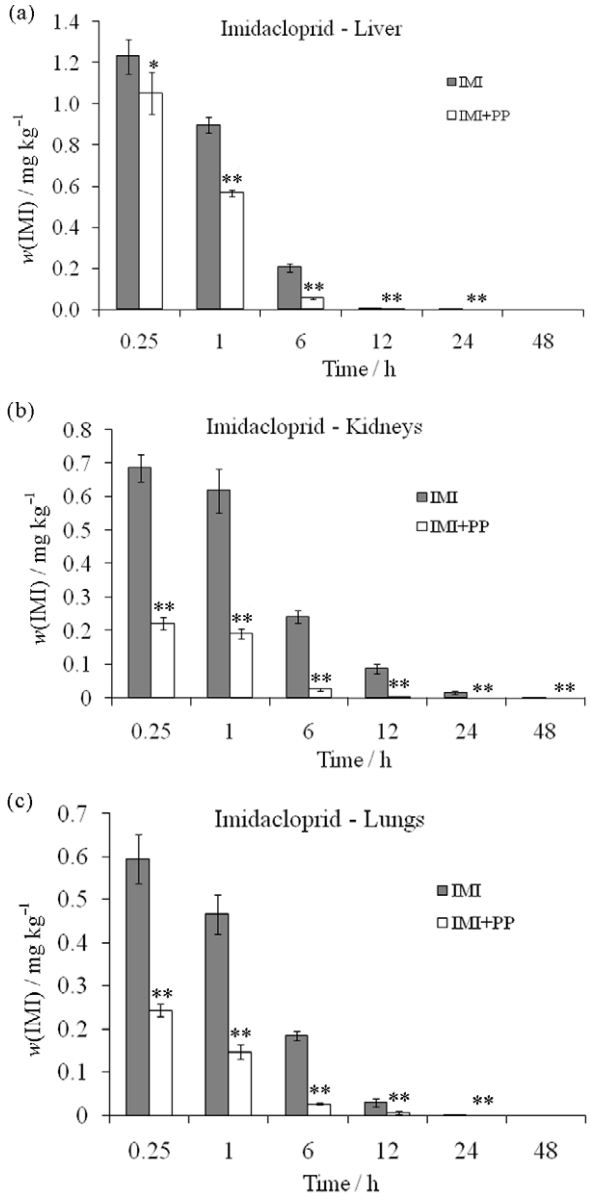

Figure 2. Imidacloprid mass fraction (*w*) in the liver (a), kidneys (b) and lungs (c) at different time intervals. Results are expressed as mean ± standard deviation (n = 4–6 mice per group).(*) $P<0.05$: statistically significant difference compared to the IMI group. (**) $P<0.01$: statistically significant difference compared to the IMI group.

sage of 6-CNA through the liver and lungs and suggests a high rate of renal excretion of IMI as found by Solecki[3] and Klein and Karl.[28]

In summary, in the IMI group, 48 hours after the administration, neither IMI nor 6-CNA could be detected in tested organs, while complete degradation in the (IMI + PP) group was accomplished within 24 hours.

In order to measure the degradation rates in mice organs, the decline of the IMI mass fraction as a function of time was monitored. To quantify the rate of degradation, the data were fitted to a classical first-order rate equation (Eq. (1)), which is in agreement with literature.[27,29] In both groups the degradation rate of IMI was proportional to the dose of administered pesticide, pointing to the suitability of used animal model in this study. The proposed model shows a good correlation between theoretical and experimental values in all tested organs in the IMI and (IMI + PP) groups (Figure 4 and Table I).

Two hours half-life obtained in the liver in the group treated with IMI indicates rapid degradation kinetics. Ford and Casida have found similar results, although the injected dose was 10 mg/kg with correspondingly shorter half-life.[27] However, these findings are consistent, con-

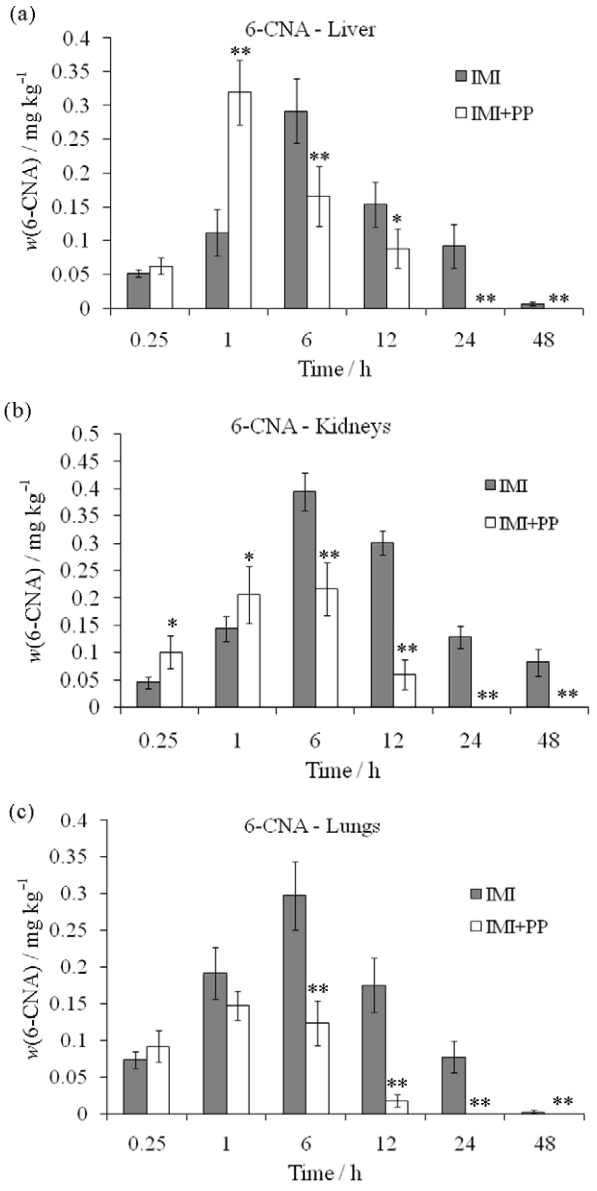

Figure 3. Mass fraction (*w*) of 6-chloronicotinic acid in the liver (a), kidneys (b) and lungs (c) at different time intervals. Results are expressed as mean ± standard deviation (n = 4–6 mice per group). (*) $P<0.05$: statistically significant difference compared to the IMI group. (**) $P<0.01$: statistically significant difference compared to the IMI group.

TABLE I. Degradation rate constants (k_t) and half-lives ($t_{1/2}$) of imidacloprid in the liver, kidneys and lungs

Organs	Group	k_t / h^{-1}	$t_{1/2}$ / h	r^2
Liver	IMI	0.3462	2.00	0.97
	IMI + PP	0.6257	1.10	0.98
Kidneys	IMI	0.1480	4.60	0.99
	IMI + PP	0.3512	1.97	0.99
Lungs	IMI	0.2534	2.70	0.99
	IMI + PP	0.3186	2.17	0.97

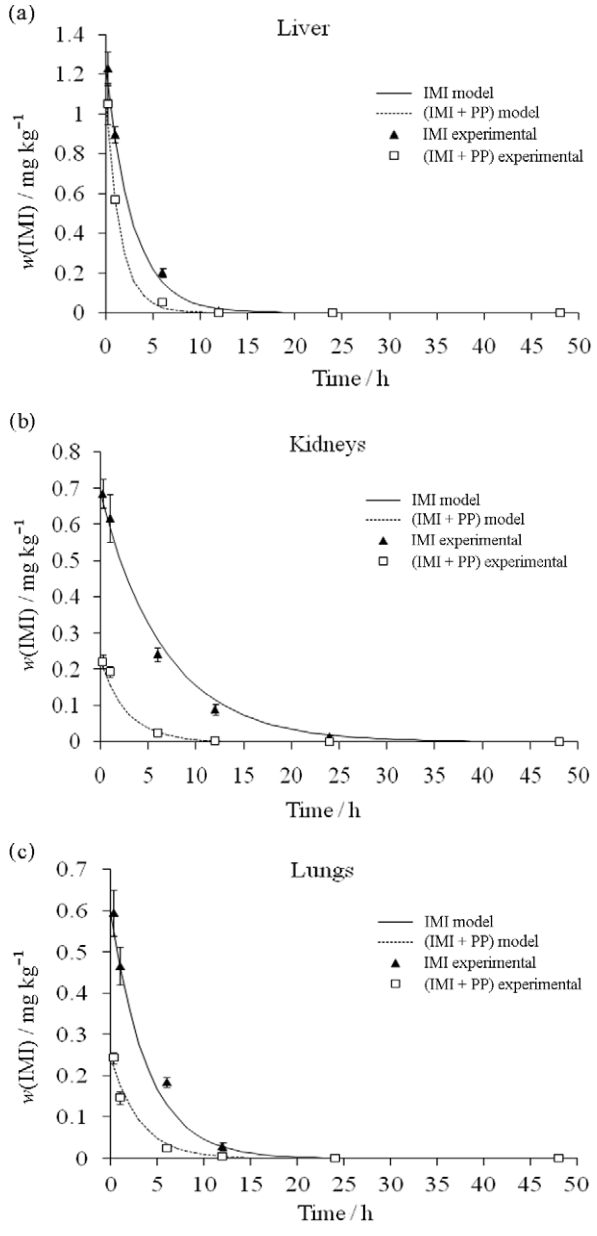

Figure 4. Proposed model of imidacloprid degradation in the liver (a), kidneys (b) and lungs (c) Results are expressed as mean ± standard deviation (n = 4–6 mice per group).

firming a scientific discussion published by EMEA. According to EMEA, IMI is rapidly distributed from the plasma into the tissues and organs of the rat, with a decrease in terminal half-life when the administered IMI dose increased.[29]

The assessment of influence of olive oil polyphenols on the IMI distribution and degradation rates in mice organs, revealed their role in the IMI metabolism. A higher rate constant and shorter half-life in the (IMI + PP) group confirms this statement (Table I).

The current study is the first report on the contributory role of olive oil polyphenols on degradation kinetics of pesticides, emphasizing a possible role of polyphenols in diminishing the toxicity of pesticides, in particular IMI.

In fact, xenobiotic-phytochemical interactions are well established. Phytochemicals have the potential to both elevate and suppress the activity of cytochromes (phase I detoxification enzymes) involved in the biotransformation of xenobiotics.[30] Among the polyphenolic compounds, some flavonoids are found to induce cytochromes, particularly CYP1A1 or CYP3A4.[31,32] The latter one has been identified as capable of mediating IMI degradation,[33] implying a possible role of olive oil phenolics in promoting the formation and excretion of IMI metabolites.

On the other hand, pesticide mediated toxicity involves excessive production of ROS,[34–38] leading to alterations in the cellular antioxidant defense system and consequently affecting susceptibility to oxidative stress. Alterations in non-enzymatic antioxidants, like gluthatione and total thiol, as well as scavenging enzymes, such as superoxide dismutase and catalase have been reported.[38–41] In addition to other low molecular weight substances, the non-enzymatic antioxidant systems includes vitamins, like vitamin C and E, suggesting that imbalance caused by oxidative stress can be replenished by dietary antioxidant intake. With respect to that assumption, the findings by Giray *et al.*[40] and Akturk *et al.*[41] have demonstrated the preventive role of vitamins E and C against pesticide induced lipid peroxidation in rats. Apart from vitamins, non-vitamin substances, such are polyphenols, can also reinforce antioxidant defence. Khan *et al.* report that pretreatment with the polyphenolic fraction of black tea increased reduced glutathione and total thiol antioxidant reserves, as well as the activities of the antioxidant enzymes, glutathione peroxidase, glutathione S-transferase and glutathione reductase after exposure to pesticides.[39] Additionally, these enzymes are important components of the phase II detoxification metabolism and thus, polyphenol treatment promotes the excretion of xenobiotics from the body.

CONCLUSION

The results of this study are in agreement with previously published findings on IMI degradation kinetics. The

data obtained in this research are particularly important, emphasizing the role of olive oil polyphenols as potent inducers of IMI degradation and their potential to lower the burden of toxic xenobiotics in animals. These findings can support the design of intervention strategies that deal with diminishing of xenobiotics toxicity. However, further studies are needed to verify the mechanisms of action by which olive oil polyphenols exert such effect.

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SAŽETAK

Kinetika razgradnje imidakloprida u organima miševa tretiranih ekstraktom polifenola iz maslinova ulja

Dalibor Broznić, Jelena Marinić, Marin Tota, Gordana Čanadi Jurešić i Čedomila Milin

Imidakloprid je insekticid širokog spektra djelovanja koji blokira nikotinski tip acetilkolinskih receptora u središnjem živčanom sustavu insekata. Međutim, imidakloprid i njegovi metaboliti mogu biti potencijalno toksični za sisavce. Stoga je u ovom radu praćena distribucija i kinetika razgradnje imidakloprida u jetri, bubrezima i plućima miševa te nastanak 6-kloronikotinske kiseline kao jednog od metabolita. Također, ispitivana je uloga polifenola iz maslinova ulja na metabolizam imidakloprida. Eksperimentalne životinje su podijeljene u tri grupe: kontrolna skupina, IMI – miševi kojima je injiciran imidakloprid (5 mg/kg) i (IMI + PP) – miševi tretirani polifenolima (10 mg/kg) tijekom sedam dana prije injiciranja imidakloprida. Nakon 48 sati imidakloprid se potpuno razgradio u IMI grupi, dok se u (IMI + PP) grupi potpuno razgradio već nakon 24 sata. Značajno veća konstanta brzine razgradnje i kraće vrijeme poluživota u (IMI + PP) grupi naglašavaju moguću ulogu polifenola maslinova ulja u smanjenju toksičnosti ovog pesticida.